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Research Article

Age features of α_2C Adrenoceptor JP-1302 selective Blockade influence on rat Myocardium inotropy

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ABSTRACT:

Nowadays the issue about the presence and the functional significance of α2-adrenergic receptors (AR) in a man's and an animal heart is the subject of intense research. Today it is known that α2-AR are present on the presynaptic membranes of adrenergic fibers membranes and on the postsynaptic membranes of myocardiocytes. The pronounced cardiovascular effects on the stimulation and the blockade of α2-AR are revealed among adult rats, the presence of chronotropic effects on the blockade of α2-AR subtype among newborn rats was shown. Further studies concerning the role of α2-adrenoceptor subtype using the selective blockers may make a significant contribution to the development of new cardio modulating and cardio protective drugs, may clarify their role in the regulation of heart functions. The obtained data will allow to complement the current understanding about regulation mechanisms of heart functioning and increase the understanding of regulatory effect adrenergic mechanisms on the contractile force of a developing organism myocardium. We studied the contractile activity of the myocardium strips in vitro. The strips of the rat atria and ventricles at different stages of early postnatal ontogenesis were attached into the tank with the working solution. In order to solve this problem the working solution was added with the selective α2C-AR blocker (JP-1302) at the concentration of 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, -10^{-9} moles. The contraction force (F) was expressed in grams (g). The study of dose dependent response of the contractile function in the ventricle and atrium myocardium among rats of various ages on the introduction of α2-adrenergic receptor blocker within the concentration range of 10^{-9}-10^{-5} moles showed that the blockade of α2A/D-AR subtypes produces the multidirectional inotropic effect among the animals of different age groups. The opposite effects of myocardial contractility during the blockade of this α-AR subtype may be associated with synthesis change, the localization and activity of various heart receptor structures which occur at different stages of postnatal ontogenesis.

Keywords: heart, chronoscope, α2-adrenergic receptors, rat.

1. INTRODUCTION

The family of adrenoceptors is the most common community in a body. They are the representatives of G-protein coupled receptors (GPCR) [10]. Nowadays nine subtypes of adrenergic receptors (AR) are determined. They are designated as follows: α1A-, α1B-, α1D-, α2A-, α2B-, α2C-, β1-, β2- and β3-AR [2, 3, 6]. All three subtypes of α2-adrenoceptors were identified in rat heart tissue by immunoblotting method. At that the densitometry measurements of strips corresponding to α2A/D-, α2B- and α2C -AR did not differ. Besides, mRNA levels of α2-adrenoceptor three subtypes found in the right and the left atrium and in the left ventricle, did not not differ significantly [4]. Protein expression of all three α2-AR subtypes was detected in single cardiomyocytes [7]. The indirect immunofluorescence microscopy with the subtype-specific antibodies and Western blot analysis showed the presence of α2A/D and α2C-adrenoceptors in a population of fetal cardiomyocyte population except for α2B-AR [9]. mRNA of all three α2-adrenoceptor subtypes were also detected in a man's heart [1]. Currently, the issue about the presence and the functional significance of α2-AR in a man's and

an animal heart is the subject of intensive research [2, 8, 12]. It is known today that α2-adrenoceptors are present on the presynaptic membranes of adrenergic fibers and on postsynaptic membranes of myocardiocytes [5, 7, 8]. α2-adrenergic receptors may cause the reduction in systemic blood pressure inhibiting sympathetic regulatory influence [8]. Also the age features of chronotropic responses to α2-AR blockade are revealed [11, 12, 13, 14, 15]. However, α2-adrenergic regulation of a heart remains understudied. Further studies concerning the role of α2-adrenoceptor subtypes using the subtype of selective blockers will help to make a significant contribution into the development of new cardio stimulating and cardio protective drugs, to clarify their role in the regulation of heart functions. The obtained data will allow to complement the current understanding about heart activity regulation mechanisms and increase the understanding about the adrenergic mechanisms of regulatory effects on the myocardium contractile force of a developing organism. The aim of this study was the examination of α2C-adrenergic receptor blockade influence on the myocardial contractility of atria and ventricles among 20, 6, 3 and 1 week old rats.

2. METHODS

The work was carried out among 20-, 6-, 3-, 1 week old outbred rats (n = 32). 20-week animals are considered as adults. The age of 6 week is prepubertal one. The formation of the adrenergic innervation of the rat heart is completed at this age. 1 week old rats do not have the adrenergic regulation of heart, and this regulation starts to develop among 3-week old rats. 25% urethane solution was used for anesthesia at the dose of 800 mg per 1 kg of a body weight. The solution was administered intraperitoneally. The contractile activity of the myocardium strips was studied using Installation Power Lab (AD Instruments, Australia) device and Statgraphics software package. The cut out strips of atria and ventricles were attached into the reservoir with the working solution in accordance with the anatomical structure of a heart. In order to record the contraction force of the myocardium strips the working solution was added with selective α2C-AR blockers (JP-1302), (Tocris), at the concentration of 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, -10^{-9} moles. The contraction force (F) was expressed in grams (g). The statistical processing and the determination of research result difference reliability according to Student's and Vulkokson's test were implemented in Microsoft Excel editor.

3.RESULTS

A negative inotropic effect is observed in the atria of 20 week old rats at in vitro experiments with α2C-AR blockade. The selective antagonist of α2C-AR JP-1302 at the concentration of 10^{-9} moles decreased the contractile force (F(g)) of isolated atrial myocardium strips by 4 ± 0,46% ($p < 0.001$) from 0,3907 ± 0,0338 g to 0,3742 ± 0,0314 g. α2C-AR blocker at the concentration of 10^{-8} moles reduced F(g) by 4 ± 0,97% ($p<0.01$) from 0,3692 ± 0,0308 g to 0,3567 ± 0,0311 g.

3The antagonist of 10^{-7} moles concentration reduced F(g) to 2 ± 0,52% ($p<0.01$), from 0,3531 ± 0,0301 g to 0,3455 ± 0,0303 g, at the concentration of 10^{-6} moles it did not provide any effect. JP-1302 at the concentration of 10^{-5} moles reduced F(g) by 3 ± 0,88% ($p<0.01$), from 0,3450 ± 0,0352 g to 0,3355 ± 0,0368 g. The ventricles of 20 week old rats demonstrate a positive inotropic effect at α2C-AR blockade. The selective antagonist α2C-AR JP-1302 at the concentration of 10^{-9} moles increased the contractile force (F(g)) of isolated ventricular myocardial strips by 7 ± 1,70% ($p<0.01$), from 0,3225 ± 0,0459 g up to 0,3447 ± 0,0488 g. The blocker α2C-AR at the concentration of 10^{-8} moles increased F(g) up to 7 ± 1,77% ($p<0.01$), from 0,3423 ± 0,0490 g to 0,3643 ± 0,0506 g. The antagonist of the concentration 10^{-7} moles increased F(g) by 3 ± 1,16% ($p<0.05$), from 0,3935 ± 0,0537 g to 0,4013 ± 0,0537 g, at the concentration of 10^{-6} moles F(g) was increased by 4 ± 1,04% ($p<0.01$), from 0,3863 ± 0,0515 g to 0,3985 ± 0,0520 g. JP-1302 at the concentration of 10^{-5} moles does not make an influence on the ventricular myocardium contraction force (Figure 1).

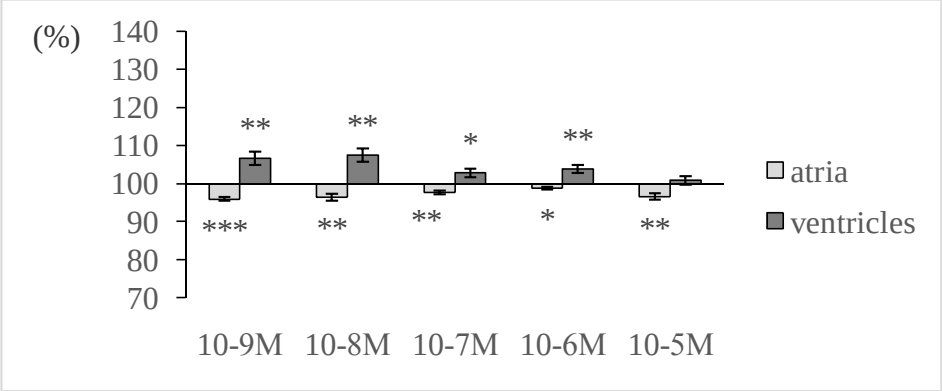

Fig. (1). The influence of α2C-AP blockade on the contraction force of the myocardium strips among 20 week old rats. Y-axis is the force of myocardial strip contraction (F, %), X-axis is the concentration of the antagonist (s, moles).

Note: * - the reliability in comparison with baseline values: p<0.05, ** - the reliability in comparison with baseline values: p<0.01, *** - the reliability in comparison with baseline values: p<0.001

The blockade of α2C-AR among 6 week old rats in the atrium and ventricle myocardium makes a multidirectional effect. The selective antagonist α2C-AR JP-1302 at the concentration 10^{-9} moles increased the contractile force (F(g)) of isolated atrial myocardium strips by 3 ± 0,74% (p<0.01), from 0,2612 ± 0,0333 g up to 0,2685 ± 0,0332 g, the blocker α2C-AR in the concentration of 10^{-8} and 10^{-7} moles did not lead to the change of F(g) from the initial value. 10^{-6} mole concentration antagonist reduced F(g) by 2 ± 0,56% (p<0.01), from 0,2574 ± 0,0266 g to 0,2511 ± 0,0255 g. JP-1302 in the concentration of 10^{-5} moles reduced F(g) by 5 ± 0,88% (p<0.01), from 0,2281 ± 0,0268 g to 0,2185 ± 0,0268 g. The selective antagonist α2C-AR JP-1302 in the concentration of 10^{-9} moles increased the contractile force (F(g)) of ventricular myocardium isolated strips by 9 ± 2,08% (p<0.01), from 0,3020 ± 0,0290 g up to 0,3298 ± 0,0320 g. The blocker α2C-AR at the concentration of 10^{-8} moles reduced F(g) by 2 ± 0,68% (p<0.05), from 0,3562 ± 0,0452 g to 0,3503 ± 0,0459 g. 10^{-7} mole concentration antagonist reduced F(g) by 2 ± 0,81% (p<0.05), from 0,3104 ± 0,0218 g to 0,3030 ± 0,0207 g, at the concentration of 10^{-6} moles F(g) was increased by 2 ± 0,50% (p<0.01), from 0,3047 ± 0,0205 g to 0,3109 ± 0,0197 g. The selective antagonist at the concentration of 10^{-5} moles reduced F(g) by 4 ± 0,69% (p<0.001), from 0,3385 ± 0,0221 g to 0,3251 ± 0,0218 g (Figure 2).

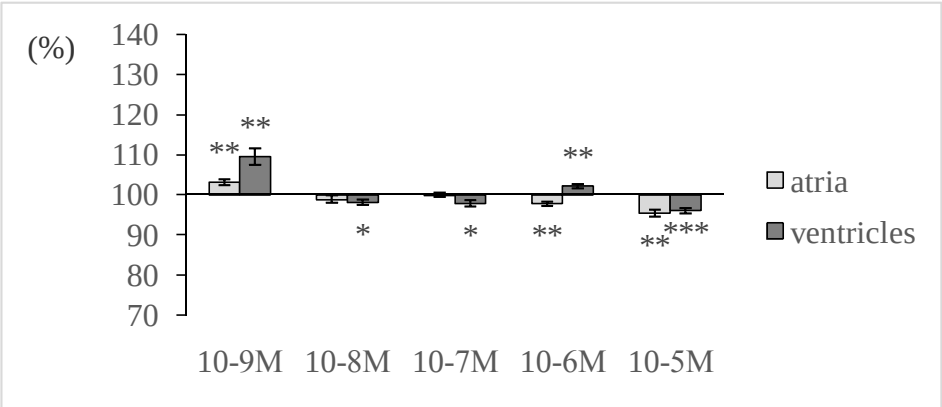

Fig. (2). The influence of α2C-AR blockade on the myocardium contraction strips among 6 week old rats. Y-axis - the myocardial strip contraction force (F, %), X-axis - antagonist concentration (s, moles).

Note: * - the reliability compared to baseline values: p<0.05, ** - the reliability compared to baseline values: p<0.01, *** - the reliability compared to baseline values: p<0.001

The experiments with α2C-AR blockade among 3 week old rats in the atrium and ventricle myocardium causes a multidirectional effect. The selective antagonist α2C AR JP-1302 at the concentration of 10^{-9} moles decreased the contractile force (F(g)) of isolated atrial myocardium strips by 7 ± 0,91% (p<0.081), from 0,1493 ± 0,0055 g to 0, 1,390 ± 0,0097 g. α2C-AR blocker at the concentration of 10^{-8} moles reduced F(g) by 5 ± 0,63% (p<0.001), from 0,1482 ± 0,0102 g to 0,31413 ± 0,0097 g. 10^{-7} mole antagonist concentration reduced F(g) by 4 ± 0,92% (p<0.01), from 0,1461 ± 0,0108 g to 0,1405 ± 0,0105 g, at the concentration of 10^{-6} moles it reduced F(g) by 3 ± 1,18% (p<0.05), from 0,1438 ± 0,0100 g to 0,1392 ± 0,0106 g. JP-1302 at the concentration of 10^{-5} moles increased F(g) by 12 ± 1,89% (p<0.001), from 0,1423 ± 0,0106 g to 0,1605 ± 0,0137 g. The selective antagonist α2C-AR JP-1302 at the concentration of 10^{-9} moles increased the contractile force (F(g)) of isolated ventricular myocardial strips by 8 ± 1,16% (p<0.001), from 0,2366 ± 0,0384 g to 0, 2540 ± 0,0392 g. α2C-AR blocker made no effect at the concentration of 10-8 moles. 10^{-7} mole concentration antagonist increased F(g) by 3 ± 1,68% (p>0.05), from 0,2780 ± 0,0495 g to 0,2857 ± 0,0511 g, at the concentration of 10^{-6} moles it increased F(g) by 4 ± 1,16% (p<0.05), from 0,3065 ± 0,0418 g to 0,3194 ± 0,0446 g. The selective antagonist at the concentration of 10^{-5} moles increased F(g) by 8 ± 1,99% (p<0.01), from 0,2645 ± 0,0389 g to 0,2839 ± 0,0389 g (Figure 3).

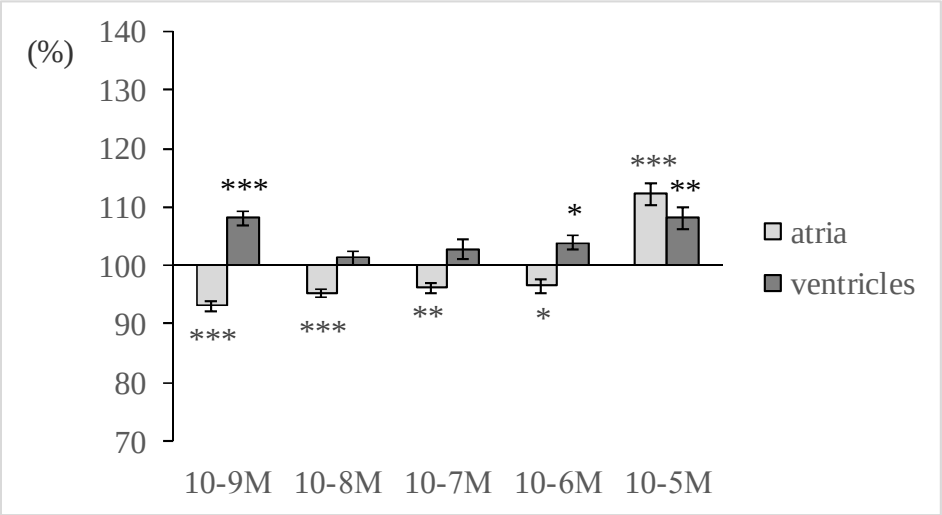

Fig. (3). The influence of α2C-AR blockade on the myocardium contraction strips among 3 week old rats. Y-axis - the myocardial strip contraction force (F, %), X-axis - antagonist concentration (s, moles).
Note: * - the reliability compared to baseline values: p<0.05, ** - the reliability compared to baseline values: p<0.01, *** - the reliability compared to baseline values: p<0.001

The experiments with α2C-AR blockade among 1 week old rats in the atrium and ventricle myocardium causes a multidirectional effect. The selective antagonist α2C AR JP-1302 at the concentration of 10^{-9} moles decreased the contractile force (F(g)) of isolated atrial myocardium strips by 3 ± 1,78% (p>0.05), from 0,0465 ± 0,0050 g down to 0,0454 ± 0,0054 g. The blocker α2C-AR at the concentration of 10^{-8} moles increased F(g) by 8 ± 1,51% (p <0.01), from 0,0454 ± 0,0069 g to 0, 0485 ± 0,0069 g. 10^{-7} mole concentration antagonist reduced F (g) by 6 ± 0,97% (p<0.001), from 0,0553 ± 0,0043 g to 0,0520 ± 0,0044 g, at the concentration of 10^{-6} moles it increased F(g) by 7 ± 1,10% (p<0.001), from 0,0533 ± 0,0042 g to 0,0569 ± 0,0043 g. JP-1302 in the concentration of 10^{-5} moles increased F(g) by 26 ± 4,22% (p<0.001), from 0,0525 ± 0,0046 g to 0,0669 ± 0,0071 g. The selective antagonist α2C AR-JP-1302 at the concentration of 10^{-9} moles had no effect on the contractile force (F(g)) of ventricular myocardium isolated strips. α2C-AR at the concentration of 10^{-8} moles reduced F(g) by 4 ± 1,52% (p <0.05), from 0,1107 ± 0,0115 g to 0,1059 ± 0,0103 g. 10^{-7} mole concentration

antagonist reduced F(g) by 4 ± 1,40% ($p<0.05$), form 0,1070 ± 0,0104 g to 0,1025 ± 0,0097 g, at the concentration of 10^{-6} moles it reduced F(g) by 5 ± 1,44% ($p <0.05$), from 0,0870 ± 0,0133 g to 0,0841 ± 0,0136 g. The selective antagonist at the concentration of 10^{-5} moles increased F(g) by 25 ± 2,39% ($p<0.001$), from 0,0788 ± 0,0141 g to 0,0978 ± 0,0171 g (Figure 4).

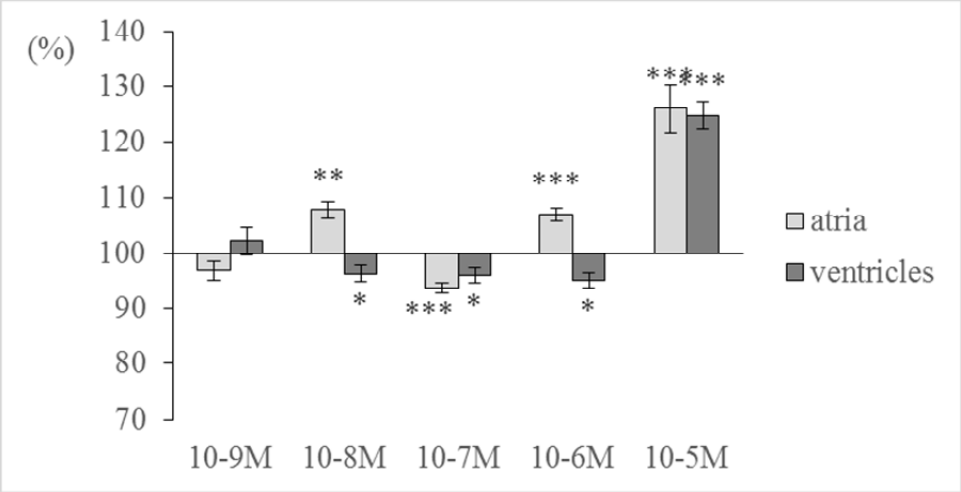

Fig. (4). The influence of α2C-AR blockade on the myocardium contraction strips among 1 week old rats. Y-axis - the myocardial strip contraction force (F, %), X-axis - antagonist concentration (s, moles).
Note: * - the reliability compared to baseline values: $p<0.05$, ** - the reliability compared to baseline values: $p<0.01$, *** - the reliability compared to baseline values: $p<0.001$

4. CONCLUSIONS

Thus, it was shown that α2C-adrenoceptor blockade causes both positive and negative inotropic effect on atrium and ventricle contractility among 20, 6, 3 and 1 week old rats.

5.SUMMARY

The performed studies showed that the selective blockade of α2C-AR makes the influence on the strength of myocardial contraction strips in all age groups among rats. At α2C-AR blockade in the atria and ventricles of all age animals the multidirectional effects were observed at the use of the blocker different concentrations. α2C-AR blockade in the atria and the ventricles leads to a pronounced positive inotropic response among 3 and 1 week old animals at the introduction of agonist highest concentration. Adult animals had the maximum effects at the introduction of α2C-AR blocker minimum concentrations. It should be noted that we also studied the chronotropic effects during selective blockade of α2C-AR adrenoceptors [11]. It was shown that the blockade of α2C-adrenoceptor makes a positive chronotropic effect among adult rats, and a negative one among 3-week-old rats. Newborn and 6-week-old rats did not show heart rate changes at the blockade of α2C adrenoceptors. Thus, the results of performed studies show a significant influence of α2C-adrenergic receptor selective blockade on the heart muscle function. It is known that α2-AR may be bound to inhibitory Gi and Go proteins and with Gs proteins, increasing adenylate cyclase activity. These results support the assumption that one of the factors determining the interaction of receptors with different G-proteins is the concentration of biologically active agents which modulate a receptor activity and its functional significance. Furthermore, there is evidence that the activation of α2-adrenoceptors by low concentration agonists leads to the decrease of intracellular cAMP level, while some higher concentration of agonist increases cAMP level [5].

CONFLICT OF INTEREST

The author confirms that the presented data do not contain any conflict of interest.

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