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CHOLINERGIC REGULATION OF THE DEVELOPING HEART CONTRACTILITY

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Abstract:

The activity of the sympathetic and parasympathetic divisions of the autonomic nervous system changes with aging. The researchers suggest that the changes in the heart activity are associated with the restructuring of the sympathetic-parasympathetic interactions of the receptor systems in the heart. Our paper describes periods of postnatal ontogenesis associated with the formation of the sympathetic cardiac innervation. The study was conducted *in vitro* with the use of Power Lab (AD Instruments, Australia) device on the strips of atrial and ventricular myocardium of rats aged 20, 8, 6, 3 and 1 weeks. The obtained results indicate that in all age groups studied the cholinoreceptor agonist – carbachol, on the background of different concentrations of a non-selective blocker of muscarinic cholinergic receptors – atropine, inhibits the inotropy of the atrial and ventricular myocardium. At the same time, the dynamics of myocardial contractility is age dependent. The findings suggest that during postnatal ontogenesis there are changes in the dynamics of myocardial contractility after administration of carbachol on the background of different concentrations of atropine. It is possible that during the main development stages of the sympathetic cardiac innervation there are changes in regulation of cardiac inotropy. Newborn rats had the least pronounced reduction of contractile force of the atrial and ventricular myocardium, and 3- and 8-week-old rats - the most pronounced.

Keywords: heart, inotropy, muscarinic cholinergic receptors, postnatal ontogenesis.

1. Introduction.

The autonomic nervous system (ANS) is a key component in the regulation of mammalian heart. Both sympathetic and parasympathetic divisions of ANS implement regulatory influence through adrenergic and cholinergic receptors of cardiac cells. The activity of the parasympathetic division is carried out through the interaction of acetylcholine (ACH) with the muscarinic cholinergic receptors (M-CR). Modern literature describes five types of muscarinic cholinergic receptors (M_1-M_5-CR) [1]. M-HR are the representatives of G-protein-coupled receptors that interact in two ways: M_1, M_3, and M_5-CR interact with $G_{q/11}$ protein, stimulating the phospholipase C, and formation of inositol triphosphate, and

diacylglycerol. M_2 and M_4-CR bind to G_i/G_o and inhibit activity of adenylate cyclase, which leads to a decrease in cAMP level [2]. Activation of additional signal system affects K^+- and Ca^{2+}-channels and activation of phospholipase A_2, phospholipase D and the protein tyrosine kinase [3; 4]. M-CR have been found in the myocardium, endocardium, T-tubules of cardiomyocytes, in the wall of coronary arteries, capillary endothelium, in the sinoatrial and atrioventricular nodes of cardiac conduction system [5]; their density is higher in the atria [6]. It is believed that the M_2-CR are predominant in mammalian and human hearts [1]. Based on findings of pharmacological studies, the researches have suggested that a third subtype of M-CR is the second largest population of M-XP in the rat cardiac myocardium [1]. Electrophysiological and pharmacological methods revealed in the the M_3-CR and M_4-CR in cardiac tissues of dogs [7]. Based on the pharmacological characteristics, the populations of M_2, M_3 or M_5-CR were found in the sheep's hearts. Radioligand binding method with the use of selective antagonists of M-CR revealed a binding site only for M_2-CR [2]. The presence of M_1, M_2, M_3 и M_5-CR was revealed by chromatographic protein analysis. Another group of researchers also confirmed the presence of mRNA M_1-M_5-CR in the atria and ventricles of the human heart. [8]. There is a great number of studies in literature that prove an important role of M_3-CR in the regulation of both chronotropy and inotropy of heart [9, 10]. Performing an adaptive function, M_2-CR reduce the frequency and strength of heart contractions [11], thereby adapting the heart to changing environmental conditions. Cholinergic activation via M_3-CR can ease a myocardial hypertrophic response being triggered by angiotensin II [12]. M_3-CR activation may increase the heart rate. M_4-CR modulate various ionic currents, providing the repolarization phase [13]. For many years, the researches have been interested in age-related features of regulating mechanisms of cardiac activity. It has been shown that the parasympathetic innervation of the heart occurs far before birth, and the sympathetic one develops later [14]. Expression of certain families of M-CR in the mammalian heart during ontogeny is different. The heart of a mature rat has expression of M_2-CR, and the atria of newborn animals-M_1 and M_2-CR. The bird's heart has expression of M_2 and M_4-CR [2]. Therefore, the study of the mechanisms of cholinergic regulation of cardiac contractility of the myocardium of rats at different stages of postnatal ontogenesis is quite relevant. Thus, objective of this research was to study the impact of the CR carbachol agonist on the background of different concentrations of a non-selective blocker M-CR atropine to the contractility of atrial and ventricular myocardium in 20-, 8-, 6-, 3- and 1-week-old rats.

2. Methods

The experiments were conducted on 20-, 8-, 6-, 3- and 1-week-old outbred rats. These age groups were selected based on the basic stages of formation of the innervation of the cardiovascular system of rats. The heart of newborn rats has

no sympathetic innervation, 3-6 week-old animals are characterized by the beginning of the development of the sympathetic cardiac innervation, 6-week-olds - by its completion, 8week-olds - by puberty, and 20-week-old animals have mature innervation of the heart [14].

Myocardial contractile activity in the *in vitro* experiment was studied on strips of the atria and ventricles. The isolated heart was placed in a tray with a process solution, and the myocardium strips of 3.2 mm long and 0.8-1.0 mm in diameter were cut out from the right atrium and right ventricle in accordance with the anatomical structure of the heart. Slices were placed vertically in the reservoir (V=20 ml) with the carbogen-oxygenated process solution. The upper end of the slice was attached to stainless rod connected to the voltage meter, and the lower end - to the rubber block. The preparation was stimulated with an electrical signal through two silver electrodes using ESL–2 stimulator (Russia) at 10 and 6 stimuli per min for the 1-, 3- and 6 - 8, 20-week-old animals, respectively, 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 minutes, during which an optimal voltage was gradually applied to the muscle fibers. At the end, the initial parameters of contraction were recorded for 5 minutes, then for 10 minutes with the addition of the specific blocker to the process solution, and for 21 minutes with the addition of agonist. A non-selective blocker of M-CR atropine was added at concentrations of 10^{-6}-10^{-3}M, agonist of CR carbachol – at a concentration of 10^{-5}M. Studying a dose-dependent effect of CR agonist carbachol (10^{-9}-10^{-5}M) on inotropy of myocardium of atria and ventricles of all age groups of rats revealed that carbachol at a concentration of 10^{-5}M causes significant negative inotropic effect [15]. The obtained results were processed in Chart 5.3 program, using Power Lab (AD Instruments, Australia), with force sensor MLT 050/D. Statistical analysis and identification of the reliability of the research results by Student t-test were performed in Microsoft Excel editor.

3. Results

Administration of carbachol in addition to atropine (10^{-6}M) reduced the contractile force of atrial myocardium in 20-week-old rats from 0.591±0.098 g to 0.481±0.068 g ($p<0.01$). Adding carbachol after blockade with atropine resulted during the first minute in a slight increase in contractile force of the ventricular myocardium from 0.703±0.2 g to 0.713±0.212 g, and by the final minute of the experiment - in decrease to 0.608±0.184 g. In addition to the effect of atropine (10^{-5}M), carbachol (10^{-5}M) reduced the contractile force of atrial myocardium from 0.155±0.074 g to 0.137±0.064 g ($p<0.05$), and ventricular myocardium - from 0.353±0.188 g to 0.268±0.151 g ($p<0.05$) by the 21st minute of the experiment. Adding carbachol after M-CR blockade (10^{-4}M) caused reduction in the contractile force of atrial myocardium from 0.387±0.057 g to 0.34±0.047 g ($p<0.01$), and ventricular myocardium from 0.475±0.136 g to

0.345±0.097 g ($p<0.01$). Adding carbachol after blockade with atropine (10^{-3}M) caused reduction in the contractile force of the atrial myocardium from 0.427±0.212 g to 0.19±0.108 g ($p<0.05$) (Fig. 2), and ventricular myocardium from 0.372±0.191 g to 0.191±0.105 g ($p<0.05$).

Adding the M-CR agonist after atropine (10^{-6}M) caused reduction in the contractile force of the atrial myocardium from 0.494±0.176g to 0.234±0.124 g ($p<0.05$), and ventricular myocardium from 0.492±0.175 g to 0.242±0.128 g ($p<0.05$) in 8-week-old rats. Adding carbachol in the process solution after the blockade with atropine (10^{-5}M) caused reduction in the contractile force of the atrial myocardium from 0.31±0.18 g to 0.221±0.141 g ($p<0.05$), and ventricular myocardium from 0.176±0.022 g to 0.142±0.028 g ($p<0.05$). Adding carbachol (10^{-5}M) on the background of atropine (10^{-4}M) caused reduction in the initial contractile force of the atrial myocardium from 0.547±0.229 g to 0.352±0.1829 g ($p<0.01$), and ventricular myocardium from 0.257±0.116g to 0.158±0.084 g ($p<0.05$). Adding carbachol on the background of the blockade with atropine (10^{-3}M) caused reduction in the contractile force of the atrial myocardium from 0.366±0.164 g to 0.168±0.1 g ($p<0.01$), and ventricular myocardium from 0.679±0.304 g to 0.338±0.26 g ($p<0.01$).

Adding carbachol (10^{-5}M) on the background of atropine (10^{-6}M) caused reduction in the contractile force of the atrial myocardium from 0.601±0.196 g to 0.488±0.159 g ($p<0.01$), and ventricular myocardium from 0.78±0.176 g to 0.675±0.157 g ($p<0.01$) in 6-week-old rats (Fig. 3). The effect of agonist (10^{-5}M) on the background of atropine (10^{-5}M) caused reduction in the contractile force of the atrial myocardium from 0.487±0.259 g to 0.398±0.231 g ($p<0.05$), and ventricular myocardium from 0.273±0.091 g to 0.213±0.082 g ($p<0.05$). Adding carbachol on the background of prior administration of atropine (10^{-4}M) caused reduction in the contractile force of the atrial myocardium from 0.689±0.158 g to 0.574±0.139 g ($p<0.05$), and ventricular myocardium from 0.649±0.179 g to 0.533±0.156 g ($p<0.05$). Adding carbachol (10^{-5}M) on the background of the blockade with atropine (10^{-3}M) caused reduction in the contractile force of the atrial myocardium from 0.845±0.209 g to 0.507±0.109 g ($p<0.05$) (Fig. 2), and ventricular myocardium from 0.982±0.187 g to 0.627±0.132 g ($p<0.01$).

The effect of carbachol on the background of atropine (10^{-6}M) caused reduction in the contractile force of the atrial myocardium from 0.26±0.0811 g to 0.105±0.04 g ($p<0.05$), and ventricular myocardium from 0.143±0.027 g to 0.062±0.008 g ($p<0.01$) in 3-week-old rats (Fig. 3). Adding agonist (10^{-5}M) on the background of M-CR blocker (10^{-5}M) caused reduction in the contractile force of the atrial myocardium from 0.122±0.039 g to 0.057±0.002 g ($p<0.05$), and ventricular myocardium from 0.079±0.021 g to 0.045±0.0145 g ($p<0.01$). Adding carbachol on the background of

atropine (10^{-4}M) caused reduction in the contractile force of the atrial myocardium from 0.277±0.078 g to 0.141±0.028 g ($p<0.05$), and ventricular myocardium from 0.324±0.108 g to 0.279±0.113 g ($p<0.01$). Adding carbachol on the background of the blockade with atropine (10^{-3}M) caused reduction in the contractile force of the atrial myocardium from 0.424±0.066 g to 0.187±0.026 g ($p<0.01$), and ventricular myocardium from 0.206±0.016 g to 0.072±0.012 g ($p<0.01$) (Fig. 3).

Adding carbachol (10^{-5}M) to the process solution on the background of the blockade with atropine (10^{-6}M) caused in newborn rats the slight changes in the contractile force of the strips of the atrial myocardium, and reduced the contractile force of the ventricular myocardium from 0.217±0.015 g to 0.2±0.02 g ($p<0.01$). Adding carbachol on the background of the blockade with atropine (10^{-5}M) caused reduction in the contractile force of the atrial myocardium from 0.127±0.038 g to 0.120±0.037 g ($p<0.05$), and ventricular myocardium from 0.162±0.021g to 0.153±0.03 g ($p<0.05$).

Carbachol on the background of atropine (10^{-4}M) caused reduction in the contractile force of the atrial myocardium from 0.243±0.065 g to 0.23±0.058 g during the 7th minute. The contractile force of the ventricular myocardium reduced from 0.504±0.076 g to 0.473±0.075 g ($p<0.05$).

Carbachol after the blockade with atropine (10^{-3}M) caused reduction in the contractile force of the atrial myocardium from 0.271±0.028 g to 0.19±0.05g ($p<0.05$) (Fig. 2).

The maximum reduction in the contractile force of the ventricular myocardium from 0.174±0.009 g to 0.087±0.0219 g ($p<0.05$) was observed during the 21st minute (Fig. 3).

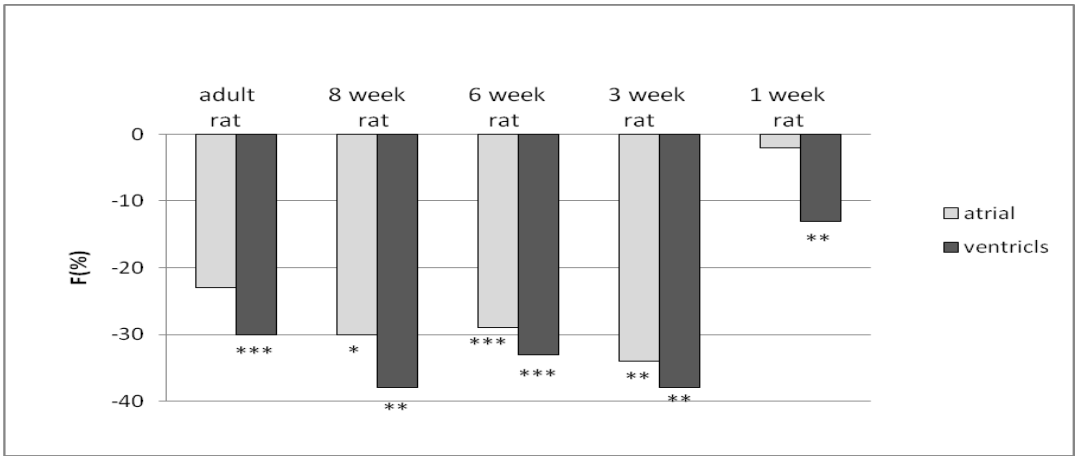

Fig. (1). Effect of carbachol (10^{-5}M) on the inotropy of the atrial and ventricular myocardium in rats. Y-axis – myocardium strips contractile force (F, %), X-axis – animal age (week). Note: * - data reliability as compared with initial values: p<0.05, ** - data reliability as compared with initial values: p<0.01, * - data reliability as compared with initial values: p<0.001**

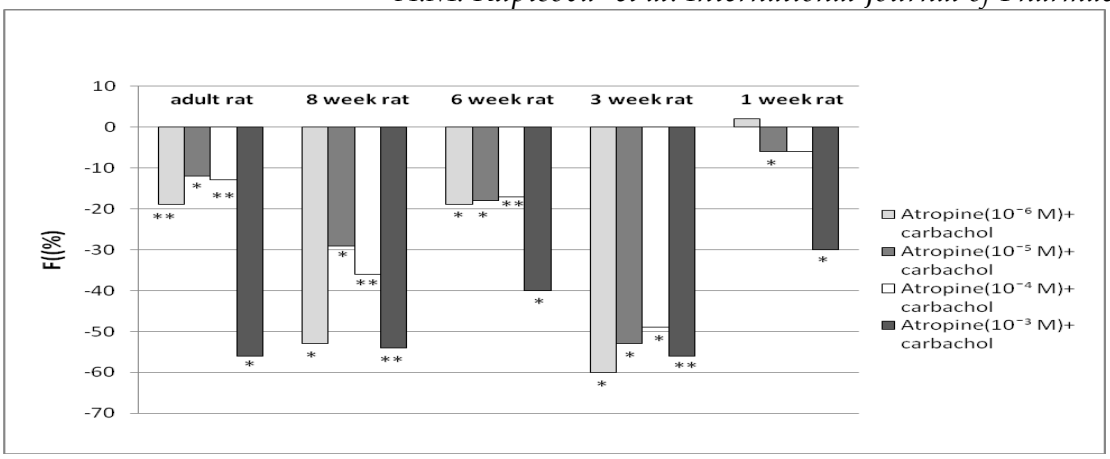

Fig. (2). Effect of carbachol (10^{-5}M) on the background of different concentrations of atropine (10^{-6}-10^{-3}M) on the inotropy of the atrial myocardium in rats. Y-axis – myocardium strips contractile force (F, %), X-axis – animal age (week). Note: * - data reliability as compared with initial values: p<0.05, ** - data reliability as compared with initial values: p<0.01.

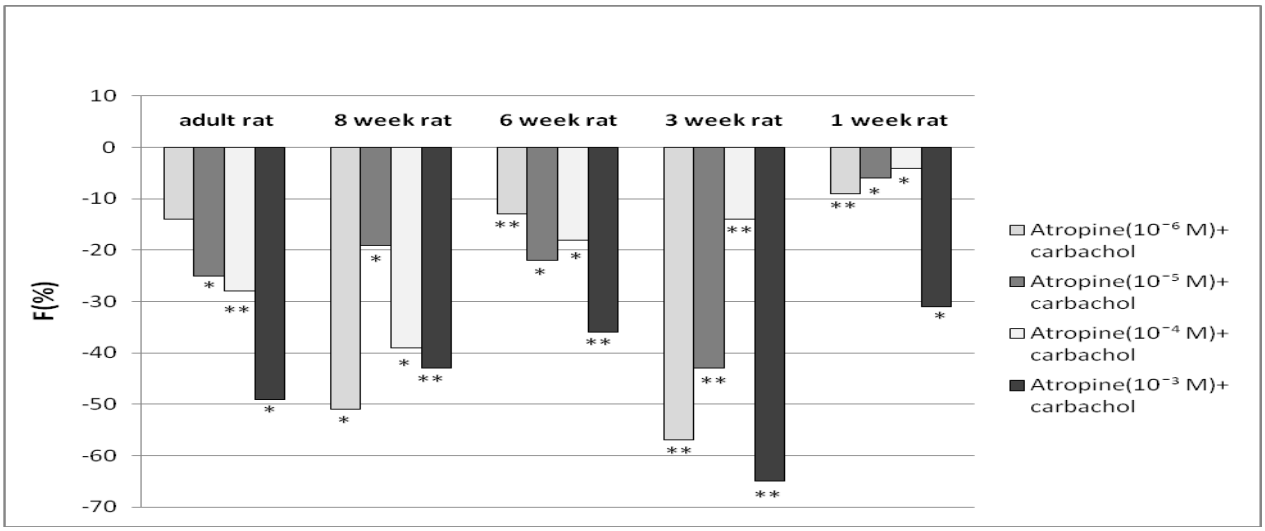

Fig. (3). Effect of carbachol (10^{-5}M) on the background of different concentrations of atropine (10^{-6}-10^{-3}M) on the inotropy of the ventricular myocardium in rats. Y-axis – myocardium strips contractile force (F, %), X-axis – animal age (week). Note: * - data reliability as compared with initial values: p<0.05, ** - data reliability as compared with initial values: p<0.01.

4. Summary

The atropine concentrations (10^{-6}-10^{-3}M) do not prevent negative inotropic effect of carbachol (10^{-5}M) in all age groups. The dynamics of myocardial contractility is age dependent.

5. Conclusion

Based on a comparative analysis of the results, we can conclude that carbachol on the background of the blockade with atropine (10^{-6}M) reduced the contractile force in the atria and ventricles of 20-week-old animals as compared with the

control administration (Fig. 1). Blockade with atropine (10^{-5}-10^{-4}M) caused no changes in the effect of carbachol, and did not exceed its action in the atria (Fig. 2). On the background of the blockade with atropine (10^{-3}M), carbachol more inhibits the contractile force in the atria and ventricles, as compared with the control action of carbachol (Fig. 1). Analysis of data of 20-, 8-, 6- and 3-week-old animals showed that carbachol on the background of blockade with atropine (10^{-6} and 10^{-4}M) more inhibits inotropy in the atria of 8-week-old animals than of 20- and 6-week-old rats, and to a lesser extent as compared with the atria of 3-week-old rats (Fig. 2). Carbachol on the background of atropine (10^{-3}M) in the atria of 20- and 8-week-old rats reduces the contractile force of the myocardium (Fig. 2), and is stronger in comparison with the 6-week-old rats and the control action of carbachol (Fig. 1). 6- and 20-week-old rats have weaker carbachol effect on atrial myocardial contractility after blockade with atropine (10^{-6}M) as compared with the control action of carbachol (10^{-5}M) (Fig. 1). The carbachol effect in the atria and ventricles on the background of atropine 10^{-6}M and 10^{-3}M is most pronounced in 3-week-old rats (Fig. 3). 1-week-old rats showed less pronounced inhibitory effect of carbachol on contractility of the atrial and ventricular myocardium on the background of all the atropine concentrations as compared with other age groups studied (Figure 2, 3). Carbachol on the background of atropine at a concentration of 10^{-3}M caused reduction in the contractile force of the strips of atrial and ventricular myocardium, and this decrease was more significant in comparison with the effect of carbachol (Figure 1).

An aspect of the scientific debate is still the question of sympathetic-parasympathetic interactions being the basis of the autonomic cardiac regulation. The activity of the sympathetic and parasympathetic divisions in the mammalian and human body changes with aging. Many researchers associate age-specific changes in the heart activity with the restructuring of the receptor systems in the heart. Our study describes periods of postnatal ontogenesis associated with the changes in the sympathetic cardiac innervation. In accordance with the theory of accentuated antagonism of the sympathetic-parasympathetic relationship, the change in the level of sympathetic activity modulates the parasympathetic effect on the heart.

After analyzing the current data, we can conclude that ACH at rest has no effect on myocardial contractility and inhibits it on the background of increase in the contractile force upon application of pharmacological agents [16]. In our experiments, carbachol caused a significant negative inotropic effect only when applied at a concentration of 10^{-5}M (Figure 1), which is substantially higher than its physiological concentration. It is possible that rats at rest produce acetylcholine from the parasympathetic preganglion cells in small quantities, and its secretion increases sharply only in special cases, such as electrical stimulation of the vagus nerve.

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