Age-Related Peculiarities of Inotropic Response of Rat Myocardium to Selective Block of M1-Cholinoreceptors

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In vitro effect of M1-cholinoreceptor blockade on the cardiac inotropic function was examined in rats aging 1, 3, 6, 8, and 20 weeks. In 1- and 3-week old rat pups, the sympathetic control of the heart has not developed, the age of 7-8 weeks being pubertal. Adult 20-week rats were used as the controls. In rats of all age groups, preliminary blockade of M1-cholinoreceptors did not prevent the inhibitory effect of carbacholine on contractility of the atrial and ventricular myocardium. The inhibitory effect of pirenzepine on the contractile force of ventricular myocardium was revealed in 6-week rats.

Key Words: *heart; inotropy; muscarinic cholinoreceptors; rat; postnatal ontogeny*

The parasympathetic regulatory influences on the heart are mediated via M2-cholinoreceptors (M2-ChR) coupled with Gi-proteins and K-channels [9]. Recent studies have demonstrated the involvement of other types of muscarinic cholinoreceptors in the regulation of cardiac activity [1,4]. Five subtypes of muscarinic cholinoreceptors (M1-M5) are now described with the corresponding encoding genes. They are subdivided into two major groups depending on the mechanism of signal transduction: M1-, M3-, and M5-ChR are coupled with Gq/11, while M2- and M4-ChR are coupled with Gi/o protein [6].

It is believed that M2-ChR dominate in human heart [7]. Stimulation of M2-ChR leads to bradycardia and induces a negative inotropic effect in isolated atrial strips. In cardiac ventricles, the negative inotropic effect induced by stimulation of M-ChR was observed only against the background of increased contractility (elevated cAMP concentration, stimulation of β-adrenoreceptors, or phosphodiesterase inhibition) [15].

The *in vivo* studies revealed age-specific effects of selective and unselective blockade of M-ChR on chronotropy of rat heart [2,4]. Low doses of atropine and pirenzepine can produce a negative chronotropic effect [11-13]. It can be hypothesized that the peculiarities of the control of the myocardial contractile activity result from interaction of acetylcholine with different subtypes of M-ChR, activation of signaling systems, and modulation of various effectors [8,10,14]. Numerous animal experiments demonstrated age-related changes in activity of cardiac muscarinic receptors. However, available data are contradictory, because some researchers reported elevation in the number and functional sensitivity of muscarinic receptors with age, while others demonstrated opposite changes of these parameters [5]. Thus, the study of the effects of selective blockade of various subtypes of M-ChR on cardiac inotropy at different stages of the postnatal ontogeny seems to be very important.

Our aim was to examine the effect of selective blockade of M1-ChR on contractility of myocardium isolated from 1-, 3-, 6-, 8-, and 20-week-old rats.

MATERIALS AND METHODS

The study was carried out on 1-, 3-, 6-, 8-, and 20-week-old random-bred rats (n=50). The rats were intraperitoneally anesthetized with urethane (1000 mg/kg, 25% solution). The isolated heart was placed in a

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bath with working solution and connected to a stimulator via two electrodes. Myocardial strips 2-3 mm in length and 0.8-1.0 mm in diameter were cut from the right atrium and right ventricle.

The preparation was immersed vertically into a chamber (20 ml) perfused with oxygenated carbogen (97% O_2, 3% CO_2) at room temperature. The upper end of the strip was fixed to a stainless rod connected to the strain gage, while its lower end was attached to a rubber plate. The strip was stimulated using an ESL-2 electric stimulator via two silver electrodes. The stimulation parameters were as follows: pulse amplitude 10 mV, duration 5 msec, and frequency 6-10 min^{-1}. Initially, the immersed strips have been conditioned for 40-60 min to optimize the tension. The optimal tension corresponded to the critical stretch beyond which a decrease in contraction force *F* was observed. After termination of the conditioning period, the initial contraction parameters were recorded over 5 min. Pirenzepine, a selective blocker of M1-ChR, was applied in a concentration of 10^{-6} M, and carbacholine, a non-selective agonist of ChR, was used in a concentration of 10^{-5} M. Contraction force (F) was measured in grams.

The data have been processed using Chart 5 and Statgraphics software (Power Lab platform; AD Instruments). Statistical analysis and evaluation of difference significance were performed using Student's *t* and Wilcoxon tests (Microsoft Excel).

RESULTS

For evaluation of the role of M1-ChR in myocardial contractility in rats of different age, selective M1-ChR blocker pirenzepine and non-selective ChR agonist carbacholine were added to the perfusion solution. Pirenzepine produced no significant changes in the contractile force of atrial and ventricular strips isolated from 20-week rats. When applied after blockade of M1-ChR, carbacholine decreased the contractile force of ventricular and atrial strips from 0.25±0.04 to 0.16±0.03 g ($p<0.01$) and from 0.35±0.17 to 0.25±0.12 g ($p<0.05$, Fig. 1, 2), respectively.

In 8-week rats, pirenzepine produced no significant changes in the contractile force of atrial and ventricular strips. Application of carbacholine after blockade of M1-ChR decreased the contractile force of atrial and ventricular strips from 0.42±0.19 to 0.25±0.13 g ($p<0.05$, Fig. 1) and from 0.12±0.04 to 0.07±0.02 g ($p<0.05$, Fig. 2), respectively.

In 6-week rats, pirenzepine significantly decreased the contractile force of ventricular strips from 0.11±0.03 to 0.10±0.03 g ($p<0.05$), but produced no effect on the contractile force of the atrial strips. Application of carbacholine against the background of M1-ChR blockade with pirenzepine decreased the contractile force of atrial and ventricular strips from 0.26±0.06 to 0.15±0.04 g ($p<0.05$, Fig. 1) and from 0.10±0.03 to 0.07±0.02 g ($p<0.01$, Fig. 2), respectively.

In 3-week rats, pirenzepine produced no significant changes in the contractile force of atrial and ventricular strips. When applied after blockade of M1-ChR with pirenzepine, carbacholine decreased the contractile force of atrial and ventricular strips from 0.13±0.05 to 0.08±0.03 g ($p<0.05$) and from 0.17±0.08 to 0.10±0.05 g ($p<0.01$), respectively.

In neonatal rat pups, pirenzepine produced no significant changes in the contractile force of atrial and ventricular strips. When applied after blockade of M1-ChR with pirenzepine, carbacholine decreased the contractile force of atrial and ventricular strips from 0.08±0.02 to 0.07±0.03 g ($p<0.01$, Fig. 1) and from 0.013±0.02 to 0.09±0.02 g ($p<0.01$, Fig. 2), respectively.

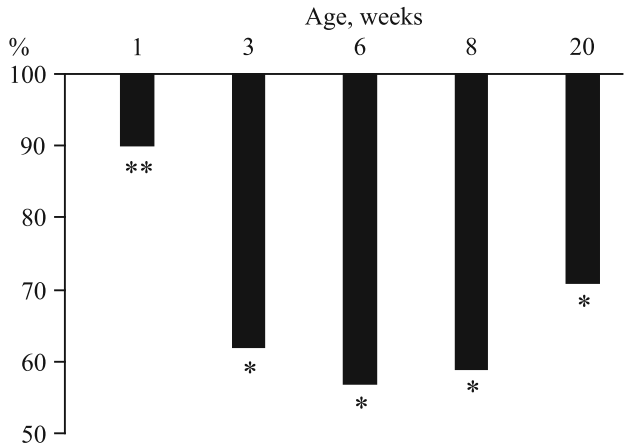

Fig. 1. Effect of carbacholine on contractile force (F) developed by rat atrial preparation preconditioned with pirenzepine. Here and in Fig. 2: *$p \leq 0.05$, **$p \leq 0.01$ compared to the values documented prior to application of carbacholine.

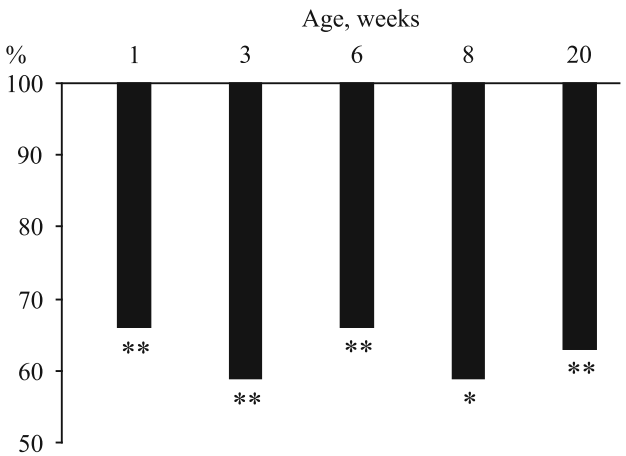

Fig. 2. Effect of carbacholine on the contractile force (F) developed by rat ventricular preparation preconditioned with pirenzepine.

Thus, M1-ChR blockade did not prevent the inhibitory action of carbacholine on contractility of the myocardium in all examined age groups, which argued against the involvement of this subtype of M-ChR in the cholinergic control of inotropy in rat heart. However, we observed a decrease in contractile force of ventricular strips treated with pirenzepine. It should be noted that this period of postnatal ontogeny is characterized with essential peculiarities in cholinergic control of the cardiac performance [3]. There are data demonstrating *in vivo* negative chronotropic effect of low pirenzepine doses [5]. It was hypothesized that this is related to inhibition of presynaptic M-ChR resulting in up-regulation of acetylcholine release. Thus, it can be hypothesized that M1-ChR are located in the presynaptic membrane, where they modulate the release of neurotransmitter.

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