PHYSIOLOGY

Age-Related Peculiarities of Contractile Activity of Rat Myocardium during Blockade of Hyperpolarization-Activated Currents

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Contractile activity of atrial and ventricular myocardial strips isolated from rats of various age was examined under conditions of blockade of non-selective hyperpolarization-activated cation currents. Addition of ZD7288, a blocker of non-selective hyperpolarization-activated cation currents, to the perfusion solution increased the contraction force of atrial and ventricular strips in 1-, 8-, and 20-week rats, but produced an opposite effect on contractile activity of atrial and ventricular strips in 3-week rats.

Key Words: *heart; inotropy; hyperpolarization-activated currents; ontogeny; rat*

In cardiac physiology, considerable attention is currently focused on the study of non-selective cation inward current activated by membrane hyperpolarization [1,4,10,15]. In atypical cardiomyocytes, these currents play the key role in the realization of the spontaneous diastolic depolarization phase of action potential [7,9,12]. In sinoatrial node cells, they are denoted as If and referred to as pacemaker or funny currents [11]. Similar non-selective hyperpolarization-activated cation currents (Ih) were found in isolated intracardiac neurons [13]. The mechanisms underlying modulation of these currents under normal and pathological conditions are currently examined [5,6,14]. If-current was recorded not only in cells of the heart conduction system, but also in working cardiomyocytes [8]. This finding prompted a hypothesis that hyperpolarization-activated currents could be involved in implementation of not only chronotropic, but also inotropic function of the heart. We previously demonstrated significant age-related peculiarities of chronotropic reaction of the rat heart *in vivo* to If blockade [2,3].

In this study, we examined the effect of If blockade on age-related peculiarities of contractile activity of rat myocardium.

MATERIALS AND METHODS

The study was carried out on 1-, 3-, 8-, and 20-week-old rats (n=7, 8, 7, and 7, respectively). The rats were anesthetized with 1000 mg/kg urethane (25% solution, intraperitoneal injection). Isolated heart was placed in a bath with working solution 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 strips were immersed vertically into a chamber (20 ml) perfused with working solution oxy-

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genated with carbogen (97% O_2, 3% CO_2) at room temperature. The upper end of the strip was fixed to a stainless rod connected to strain gage, and its lower end was attached to a rubber plate. The strips were stimulated by an ESL-2 electric stimulator via two silver electrodes. The stimulation parameters were as follows: pulse amplitude 10 mV, duration 5 msec, and repetition rate 6 min^{-1}. Initially, the immersed strips were conditioned for 40-60 min to optimize the tension. The optimal tension corresponded to a state, when further stretch increment led to a decrease in contraction amplitude (CA). After termination of the conditioning period, the initial contraction parameters were recorded over 5 min. Then the same parameters were recorded over 21 min in the presence of 0.2 μM ZD7288, a specific blocker of H-channels. CA was measured by the weight of a load in grams that the strip could lift during contraction.

The results were analyzed statistically using Student's *t* and Wilcoxon's tests.

RESULTS

In 20-week rats, the initial CA of myocardial strips isolated from the right atrium was 0.16±0.06 g. After addition of If blocker ZD7288 to the perfusion solution AC persistently increased: by 4 % ($p<0.05$) during the first minute of application and by 11, 30, and 39% by minutes 3, 10, and 20, respectively, compared to the initial values ($p<0.01$). The maximum increase in atrial CA was 41% ($p<0.05$, Fig. 1). Initial CA of ventricular strips was 0.26± 0.07 g. After application of ZD7288, CA increased by 7% over the first 10 min, by 11% ($p<0.05$) by minute 15, and then monotonously grew and surpassed the initial value by 14% to the end of the observation period ($p<0.05$, Fig. 2).

In 1-week rats, which still had no sympathetic innervation in the heart, initial CA of atrial strips was 0.06±0.03 g. After application of ZD7288, CA increased by 3% ($p<0.05$) over the first minute and by 14, 24, and 32% by minutes 5, 10, and 20, respectively ($p<0.01$, Fig. 1). The initial CA of ventricular strips was 0.035±0.007 g. By minute 5 of ZD7288 application, this parameter increased by 4% ($p<0.05$) and then gradually increased and by minute 20 surpassed the initial value by 12% ($p<0.01$, Fig. 2).

In 3-week rats, function of the cardiac sympathetic system appeared. Initial CA of atrial strips was 0.25±0.14 g. CA insignificantly decreased by 1% immediately after application of If blocker and then by 3 and 8% by minutes 5 and 15, respectively ($p<0.05$). To the end of the experiment, AC decreased by 12% ($p<0.01$, Fig. 1). Initial CA of ventricular strips from 3-week rats was 0.09±0.02 g. This parameters decreased by 3% ($p<0.01$) and 9% ($p<0.05$) by minutes 3 and 12 of ZD7288 exposure. By the end of the experiment, AC decreased by 15% ($p<0.01$, Fig. 2).

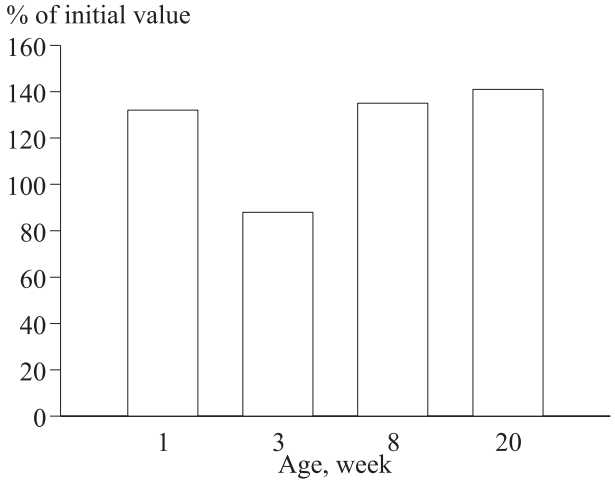

Fig. 1. Effect of If blockade on CA of atrial strips in rats of various age.

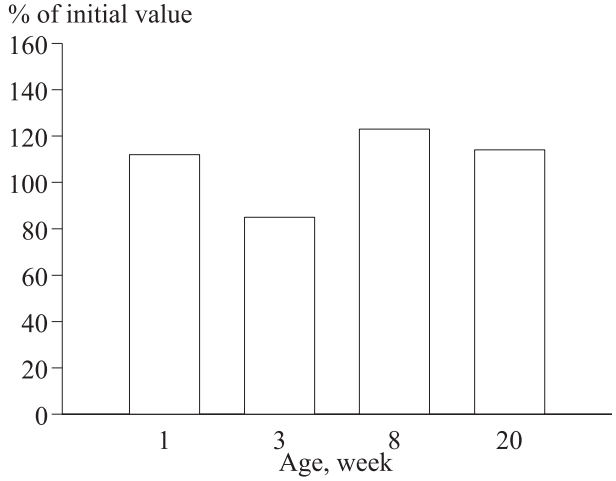

Fig. 2. Effect of If blockade on CA of ventricular strips in rats of various age.

In 8-week rats (puberty), initial CA of atrial strips was 0.23±0.05 g. By minutes 1, 5, and 15 of ZD7288 exposure, this parameter increased by 2, 8, and 16%, respectively ($p<0.05$). The maximum increment (35%) was observed on minute 16 ($p<0.01$, Fig. 1). Initial CA of ventricular strips from these animals was 0.107±0.009 g and under the effect of ZD7288 it increased by 13% ($p<0.05$) and 18% ($p<0.05$), respectively, by minutes 10 and 15. The maximum increment (23%) was observed on minute 20 ($p<0.05$, Fig. 2).

Our findings indicate the involvement of hyperpolarization-activated currents in the regulation of

myocardial contractile activity in rat atria and ventricles. The *in vitro* experiments showed that blockade of these currents potentiated atrial and ventricular contractility. The only exception was the group of rats aging about 3 weeks: in these rats, the reaction of myocardial strips dramatically differed from that in other age groups. This phenomenon can be related to the development of cardiac sympathetic innervation at this age. We previously demonstrated the existence of significant age-related peculiarities of cardiac chronotropic reaction during blockade of If currents in the rats of this age *in vivo* [2,3].

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