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Effect of If Current Blockade on Newborn Rat Heart Isolated According to Langendorff

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The study examined the effects of hyperpolarization-activated funny current (If) on HR and coronary flow in Langendorff-isolated hearts from newborn rats. Blockade of If current with ZD7288 changed the examined cardiac parameters. The blocker in a concentration of 10^{-9} M decreased HR by 26.8% ($p \leq 0.05$). In concentrations 10^{-8}, 10^{-7}, 10^{-6}, and 10^{-5} M ZD7288 produced minor differently directed effects. In a concentration of 10^{-5} M, ZD7288 reduced coronary flow in the isolated heart ($p \leq 0.01$). In other concentrations, the blocker produced no significant effects on coronary flow.

Key Words: *hyperpolarization-activated current; isolated heart; chronotropy; coronary flow; rat*

The cardiac rhythm is the most important indicator of the state of the cardiovascular system under the normal and pathological conditions [10]. Atherosclerosis, disturbances in the coronary circulation, heart failure, hypertension, and stroke are related to tachycardia irrespective of other cardiovascular pathologies [6,9]. In order to decrease HR, β-adrenoblockers and calcium channel blockers are widely used [9,11]. Remembering the limitations in the use of these drugs, a novel drug (selective blocker of If current) was proposed to decrease HR. It is currently accepted that the phase of spontaneous diastolic depolarization is associated with activation of If currents [11]. Activation (or inhibition) of If can accelerate (or decelerate) cardiac rhythm by changing the duration of diastolic depolarization.

The physiological role of HCN channels responsible for If currents in the heart and CNS is extremely important [2,7]. These channels are promising targets for novel drugs, because they regulate If currents thereby affecting cardiac rhythm and HR. The therapeutic reduction of HR in the treatment of various cardiac pathologies is an important way to improve heart functions during tachyarrhythmias [12]. The blockade of If currents modifies the electrical activity of the working ventricular cardiomyocytes by lengthening their action potential at 50-90% repolarization level [1]. In diabetic mice, blockade of If currents by ivabradine diminished apoptosis, decelerated expression, and down-regulated the action of MMP-2 thereby improving the cardiac function [3]. It was established that If current amplitude changes with age [4]. Logically, the decelerating effect of If current blockade on the HR *in vivo* is also age-dependent [14,15]. *In vitro*, blockade of hyperpolarization-activated currents with ZD7288 augmented the contractile force of isolated myocardial strips of the right atrium and ventricle of mature and newborn rats, although it attenuated this force in 3-week-old animals [13]. In ventricular cells of the old or sick animals, this current is especially pronounced at positive membrane potentials [5]. A decrease in If currents provokes spontaneous arrhythmia, which explains the need for further studies focused on detection of specific HCN subtype(s) and the fine mechanisms implicated in genesis of cardiac arrhythmias [8].

Our aim was to examine the *ex vitro* effects of hyperpolarization-activated currents on parameters of

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working hearts of the newborn rats under the conditions of Langendorff heart perfusion technique.

MATERIALS AND METHODS

The experiments were carried out *ex vivo* on random-bred newborn (7-day-old) albino rats (n=7), which had no sympathetic innervation of the heart. The control group comprised the mature rats aging 20 weeks (n=7) known to have a developed system of autonomic control of the heart. The rats were anesthetized with intraperitoneal urethane (800 mg/kg, 25% solution). The hearts were rapidly isolated and washed with cold Krebs–Henseleit solution at 2-5°C, thereupon they were cannulated via aorta and perfused at 37°C with oxygenated physiological solution (95% O_2, 5% CO_2) under a constant pressure of 60-65 mm Hg in a Langendorff system (AD Instruments). To calculate HR in the newborn rats, their cardiac electrograms were led with atraumatic electrodes mounted directly on the heart. The changes in coronary flow (CF) induced by pharmacological agents were also measured. The signals were recorded in a Powerlab 8/35 system (AD Instruments) operated under LabChartPro software. The blocker of hyperpolarization-activated current ZD7288 was employed at concentrations of 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, and 10^{-5} M.

The data were analyzed statistically using Student's t test at p<0.05 and summarized as $m\pm SEM$.

RESULTS

Prior to addition of 10^{-9} M ZD7288 (If current blocker) to perfusion solution, HR was 220±30 bpm. During the first minute after addition of ZD7288, HR dropped to 200±20 bpm. Them this parameter decreased to 186±18 bpm ($p\leq0.05$) by minute 5, and to 180±20 bpm ($p\leq0.05$) by minute 10. Finally (20 min), HR decreased maximally by 27% (to 160±20 bpm; $p\leq0.05$, Fig. 1). In the control group consisting of mature rats, the same concentration of the blocker significantly decreased HR by 26±6% ($p\leq0.01$, Fig. 2).

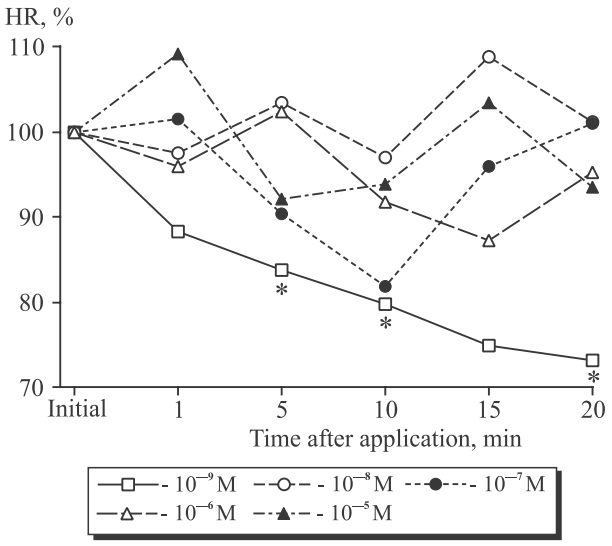

Fig. 1. Dose-dependent effect of blocker of If currents ZD7288, on HR of isolated heart of newborn rats. *p<0.05 in comparison with initial levels.

On minute 10 after application of ZD7288, CF of isolated heart decreased by 8% from 1.38±0.12 to 1.27±0.14 ml/min. In the control group of 20-week-old rats, CF decreased by 21±5% ($p\leq0.001$).

ZD7288 used in a concentration of 10^{-8} M evoked opposite and insignificant changes in HR. On minute 1, HR insignificantly changed from 214±30 to 210±20 bpm, and on minute 5, it increased to 220±30 bpm. On minute 10, HR decreased to 210±40 bpm and then increased to 230±30 bpm on minute 15. Finally, HR returned to the initial level on minute 20. In the control group consisting of mature rats, the same concentration of If current blocker also evoked opposite changes in

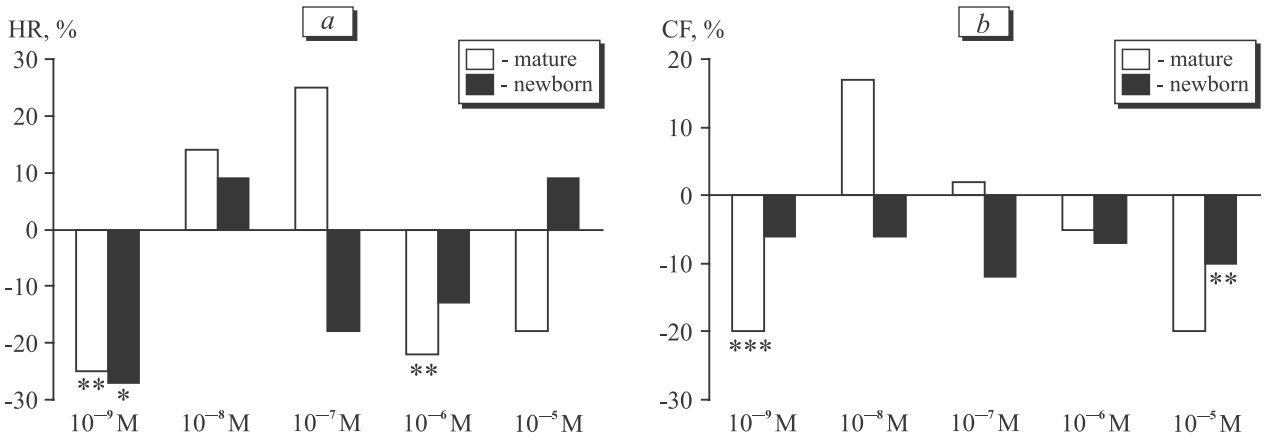

Fig. 2. Effects of ZD7288, a blocker of If currents, on parameters of hearts isolated from mature (20-week-old) and newborn (1-week-old) rats. Shown are the maximal effect on HR (*a*) and CF (*b*). *p<0.05, **p<0.01, ***p<0.001 relatively to initial values.

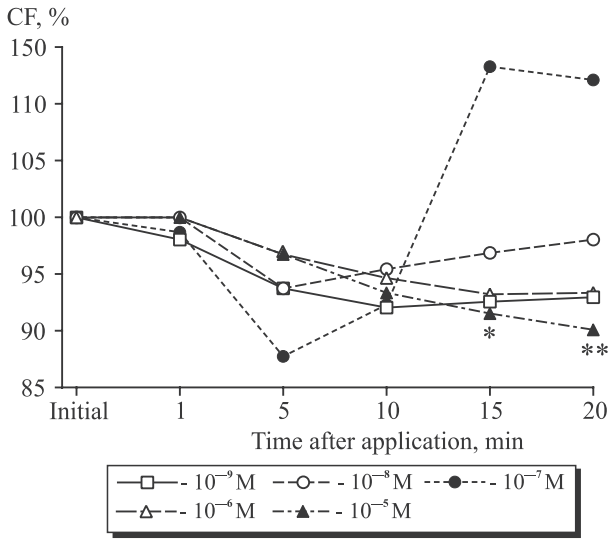

Fig. 3. Dose-dependent effect of ZD7288, a blocker of If currents, on CF of isolated heart of newborn rats. *p<0.05, **p<0.01 in comparison with initial levels.

HR. Neither in newborns nor in mature rats, significant changes in CF were observed under these conditions.

Similarly, 10^{-7} M of ZD7288 produced no significant effect on HR of the newborn rats. On minute 1, HR insignificantly increased from 210±40 to 220±40 bpm; thereupon it decreased insignificantly by 20% down to 170±20 bpm on minute 10 and increased to 220±40 bpm on minute 20. In control group of mature rats, the same concentration of blocker (10^{-7} M) also evoked the opposite variations of HR.

Blockade of If currents in isolated heart with 10^{-7} M of ZD7288 insignificantly increased CF. During 5 minutes, CA decreased insignificantly by 12.2% from 1.3±0.5 to 1.1±0.5 ml/min, thereupon it increased by 13% to 1.5±0.6 ml/min on minute 15. In mature rats, the same concentration of ZD7288 (10^{-7} M) also produced the opposite and insignificant changes in CF.

In newborn rats, the greater concentration of ZD7288 (10^{-6} M) evoked the opposite and insignificant changes in HR. On minute 1, HR somewhat decreased from 220±30 to 210±30 bpm but returned to 220±40 bpm on minute 5. On minute 10, the blocker diminished HR by 8% down to 200±30 bpm. The maximal drop in HR by 13% to 190±30 bpm was observed on minute 15. On final minute 20, there was a positive trend to restore HR. In contrast to the newborn rats, this concentration of ZD7288 significantly decreased HR in mature rats by 22±8% ($p \leq 0.01$, Fig. 2).

ZD7288 in a concentration of 10^{-6} M insignificantly decreased CF on minute 15 after application by 6.7% (from 1.4±0.5 to 1.3±0.5 ml/min). In the hearts of mature rats, CF changed insignificantly.

Interestingly, 10^{-5} M ZD7288 produced no significant effect on HR in the newborn group. On minute 1 after application of ZD7288, we observed insignificant tachycardia: HR increased by 9.2% from 180±30 to 200±30 bpm. In the following, HR decreased to 170±50 bpm on minute 10 and increased to 190±40 bpm on minute 15 terminating by a 6.5% decrease to 170±40 bpm at the end of observation period. At this concentration of ZD7288, the mature hearts of rats demonstrated insignificant bradycardia.

In newborn rats, ZD7288 (10^{-5} M) decreased CF by 9.9% from 1.5±0.6 to 1.4±0.6 ml/min ($p \leq 0.01$, Fig. 3). In the group of mature rats, the same concentration of ZD7288 diminished CF by 20±11%.

The present data paradoxically showed that blockade of hyperpolarization-activated currents in the newborn rats resulted in significant decrease of HR only when ZD7288 was applied at minimal concentration of 10^{-9} M ($p \leq 0.05$). At other concentrations, it exerted insignificant and opposite effects on HR. When used in the newborn rats at concentrations of 10^{-8}-10^{-5} M, the blocker provoked either bradycardia or tachycardia. Probably, the high concentrations of ZD7288 activated the intracardiac regulator mechanisms resulting in attempts of the heart to maintain the optimal rhythm by increasing or decreasing HR. In contrast to mature rats, the newborn ones demonstrated significant bradycardia only at minimal concentration (10^{-9} M) of the blocker (Fig. 2), whereas other tested concentrations induced the opposite effects on HR. These results agree with the data describing the effect of If current blockade on the cardiac rhythm [8,10,14]. Importantly, this blockade affected not only HR in the newborn rats, but also the tone of their coronary vessels and consequentially the blood supply to the heart. Probably, HCN channels are also available in the smooth muscle cells of the blood vessels, where they can be implicated in the control of vascular tone. The presence of corresponding If currents was demonstrated in the working cardiomyocytes [4]. Previously we showed that blockade of If currents changed the contractile force of isolated myocardial strips [15] and action potential of the working cardiomyocytes [13]. Evidently, the widespread use of If current blockers in clinics promotes further studies of their effects on various properties of the heart.

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