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The Influence Of I_F-Current Blockade On The Parameters Of Action Potential Among 1-Week And 20-Week-Old Rats.

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ABSTRACT

Nowadays the currents activated by hyperpolarization are the object of close attention and study among modern physiologists. This type of currents participates in slow diastolic depolarization and is considered the sinus-atrial node current. In literary sources, much is said about the fact that I_f-currents are present in atypical cardiomyocytes. But the information about the role of currents in functional cardiomyocytes during the generation of action potential is scarce. One of the common current blockers activated by hyperpolarization is ZD7288. The purpose of this work was to reveal the effect of ZD7288 in the concentrations of 10-9 - 10-5 M on the electrical activity of the heart among 1-week and 20-week-old rats. The experiments were carried out on a microelectrode device using a standard method of action potential shift using a glass microelectrode with the resistance of 40 - 80 MΩ. In the course of the work, they calculated such parameters of the action potential as the duration of the action potential at the repolarization level of 50%, 90%, and 100%. The results of experiments showed that ZD7288 in concentrations of 10-9 M and 10-8 M did not cause significant changes in action potential parameters. The remaining concentrations cause a significant increase of the action potential duration at the repolarization level of 50%, 90%, and 100%. Consequently, the blockade of currents activated by hyperpolarization causes the increase of the action potential time parameters.

Keywords: action potential, action potential duration, the duration at repolarization level of 50% and 90%, heart, rat

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INTRODUCTION

The currents activated by hyperpolarization (I_f or "funny" currents) were discovered by the group of scientists in the seventies of the 20th century [5, 6]. Nowadays, "funny" currents are considered to be one of the currents of the sinoatrial node, which, as is known, is involved in the formation of slow diastolic depolarization [1,3,10, 11,14]. This type of current is activated at the final repolarization level at -55 mV and at that time sodium and potassium ions rush into a cell [5, 6, 11, 13; 14; 21]. The sodium part of the current predominates over the potassium one. This type of current is active most of all during diastolic depolarization and reaches its limiting value at the level of its final third [22].

This current, originally described in the myocytes of the sinoatrial node, as an internal current activated in hyperpolarization, possesses the properties suitable to generate repetitive activity and to modulate the spontaneous rate. The degree of this current activation at the end of the action potential determines the duration of slow diastolic depolarization; consequently, the frequencies of the action potential occurrence. Since I_f-currents are controlled by intracellular cAMP and thus are activated and inhibited by the stimulation of adrenergic and muscarinic receptors of the second type (M2), respectively, they are the main physiological mechanism that mediates the vegetative regulation of heart rate. Given the complexity of the cellular processes associated with rhythmic activity, an accurate quantitative assessment of the extent to which I_f-currents and other mechanisms contribute to pacing is still a matter of discussion [12]. Nevertheless, the vast amount of information collected since this type of current had been described more than 30 years ago clearly agrees to identify I_f-currents as the main participant both to create spontaneous activity and to control speed. I_f-dependent pacemaking has shifted recently from a basic, physiologically corresponding concept, as was described originally, to a practical concept that has several potentially useful clinical applications and can be valuable in therapeutically relevant conditions. As a rule, given their exceptional role in pacing, I_f-channels are ideal objects of drugs aimed at pharmacological control of heart rhythm. The molecules that can be bound specifically to I_F channels and block them can be used as pharmacological tools to reduce the heart rate with little or no side effects of cardiovascular diseases. Besides, it is now known that some mutations of HCN4 channel function disappearance (hyperpolarization - activated cyclic nucleotide gated channels - HCN 4) through which currents pass activated by hyperpolarization and which are the main constitutive subunit of I_f channels in pacemaker cells, cause rhythm disturbances today, such as, for example, inherited sinus bradycardia [12].

They also studied the effect of current blockade activated by hyperpolarization on the inotropy of rat heart [2], which caused a positive inotropic effect on the atrial and ventricular bands of rats [23]. Recently, this type of current has been detected not only in atypical, but also in the activity of atrial and ventricular myocardiocytes. The functional role of I_f-currents in these cells, which do not exhibit the property of automation, has remained questionable for a long time. Recently, new data have been obtained, suggesting the possible participation of currents activated by hyperpolarization in the operation of functional cardiomyocytes [4].

Literary sources indicate the role and the mechanism of current regulation activated by hyperpolarization in the sinus-atrial node among atypical myocardiocytes, but the issue of their role and regulation in functional myocardiocytes is still open.

The purpose of our study was the effect of I_f-current blockade on the electrical activity of adult and newborn rats.

METHODS

The subject of the study was one- and twenty-week-old mongrel white rats. The rat was sacrificed by decapitation. All experiments were conducted in compliance with all ethical norms and rules. The chest was opened, then the heart was quickly removed and placed on a working surface. Further, the right atrium was excised, without the disturbance of the sinus-atrial node. The finished micropreparation was placed in a special bath, through which the physiological solution Tyrode was passed. 15.08 g of sodium chloride (NaCl), 0.6 g of potassium chloride (KCl), 0.28 g of sodium dihydrogenphosphate (NaH_2PO_4), 0,12 g of magnesium sulfate ($MgSO_4$), 3.36 g of sodium hydrogencarbonate ($NaHCO_3$), 0.268 g of calcium chloride ($CaCl_2$), 1.8 g of

glucose were spent in 2 liters of this solution. The drug was in the stretched position in the bath. The drug was left for 20-30 minutes to stabilize the heart muscle.

The work was carried out using a standard method of removal via the microelectrode installation using a glass microelectrode. The microelectrode, with the resistance of 40 - 80 MΩ, was filled with 3M of KCl solution and placed in a special holder, which in its turn was fixed to a micromanipulator. Then, using a macro and a micro screw, the microelectrode was lowered. The signals were generated using the amplifier (the model 1600) and displayed on the oscilloscope and on the computer monitor.

The experiment results were processed using the Elph and AP_Calc_23.04.12 programs. The reliability of the obtained results was determined using the paired Student's t-criterion. The following characteristics of the action potential were analyzed: the duration of the action potential (DAP) at the repolarization of 50%, 90%, 100%. The repolarization time was calculated as the time from the peak of the action potential to 50, 90 and 100% of the action potential drop.

During the study they used the blocker of If-currents ZD7288 in the concentrations of 10^{-9} - 10^{-5} M as the pharmacological drug.

RESULTS

The laboratory mongrel rats of 1-week (newborn) 20-week-old were examined in the experiments. When ZD7288 was added at the concentrations of 10^{-9} M and 10^{-8} M, significant changes were not observed in the parameters of rats of both ages (Fig. 1, Fig. 2).

When they studied the concentration of 10^{-7} M, the initial value of the parameter dpd 100% among 20-week-old rats was 42.28571 ± 7.973169, and on the 15th minute of the experiment a significant increase of this parameter value was observed up to 52.552 ± 14.63647 (p≤0.05). During the entire experiment among adult rats, 100% DDP value increased by 24% on the 15th minute of the experiment and made 52.552 ± 14.63647 (p≤0.05) as compared to the baseline values. This concentration did not make significant changes on the other investigated indicators of the action potential among adult animals.

When they studied the concentration of 10-7 M among the rats of week-old age, the initial value of the parameter dpd50% was 8.428571 ± 2.070197, at the time of drug administration a significant increase was observed up to 9.714286 ± 2.627691 (p≤0.01), during the 7th minute of the experiment there was a significant increase to 12.42857 ± 2.878492 (p≤0.01), and up to 12 ± 2.581989 (p≤0.01) during the 15th minute of the experiment. The initial value of dpd90% was 15.85714 ± 4.336995, a significant increase was observed at the 7th and the 15th minute of the experiment and had the values of 21 ± 4.281744 (p≤0.01) and 20.28571 ± 3.450328 (p≤0,01). The initial value of dpd100% was 29 ± 4.618802, at the 7th and the 15th minute of the experiment there was a significant increase up to 33.85714 ± 4.9447342 (p≤0.01) at the 7th minute, and up to 34 ± 4.320494 (p≤0.01) at the 15th minute respectively. Thus, during the entire experiment, the maximum increase in the following parameters of the action potential was noted: ddd50% - by 47% at the 7th minute and amounted to 12.42857 ± 2.878492 (p≤0.01), dpd90% - by 32% at the 7th minute of the experiment and had the value of 21 ± 4.281744 (p≤0.01), dpd100% - by 17% at the 15th minute of the experiment and made 34 ± 4,320,494 (p≤0.01).

During the study of the concentration 10-7 M, the initial value of the parameter dpd100% among 20-week-old rats was 42.28571 ± 7.973169, and on the 15th minute of the experiment a significant increase of this parameter value was observed up to 52.552 ± 14.63647 (p≤0.05). During the entire experiment among adult rats the value of dpd100% increased by 24% on the 15th minute of the experiment and made 52.552 ± 14.63647 (p≤0.05) as compared to the initial values. As for the other investigated indicators of the action potential among adult animals, this concentration did not cause significant changes.

When they studied the concentration of 10-6 M among 20 week old rats, the initial value of dpd50% was 8.857143 ± 2.672612, and then a significant increase of this parameter was observed with the administration of the drug to 10.21429 ± 2.233404 (p≤0,01), at the 7th minute of the experiment - up to 17 ± 2,645,751 (p≤0,01), on the 15th minute - up to 17,94286 ± 3,823984 (p≤0,01). The initial value of dpd90% was 26.42857 ± 5.563486. With the introduction of the substance, and also during the 7th and the 15th minute of

the experiment, a significant increase of this parameter in the action potential was observed up to 28.28571 ± 4.846452 (p≤0.01), 35.57143 ± 4.790864 (p≤0.01), 34,71429 ± 4.461475 (p≤0.01), respectively. The initial value of the action potential parameter dpd100% among 20-week-old rats was 41.28571 ± 7.158079. During the 7th minute of the experiment, a significant increase of the action potential was observed up to 51.71429 ± 5.274602 (p≤0.01), and during the 15th minute - up to 52.42857 ± 10.37396 (p≤0.05). Thus, among adult rats, this concentration had the maximum effect on such parameters of the action potential as dpd50% - by 102% during the 15th minute of the experiment and made 17.94286 ± 3.823984 (p≤0.01), dpd90% - by 34% during the 7th minute of the experiment and made 35.57143 ± 4.790864 (p≤0.01), dpd100% - by 27% during the 15th minute of the experiment and made 52.42857 ± 10.37396 (p≤0.05).

During the study of the same concentration among week-old rats, the initial value of the action potential parameter dpd50% was 7.714286 ± 0.48795. When the substance was administered, a significant increase was observed up to 8.857143 ± 1.214986 (p≤0.05), at the 7th minute of the experiment - up to 14.14286 ± 2.115701 (p≤0.01), during the 15th minute of the experiment - up to 14,42857 ± 1.98806 (p≤0.01). The initial value of dpd90% was 13.14286 ± 1.864454. During the 7th and the 15th minute of the experiment, a significant increase of this parameter was observed up to 22.11486 ± 3.0778342 (p≤0.01), and up to 22 ± 3.366502 (p≤0.01), respectively. The initial value of dpd100% among week-old rats was 25 ± 2.081666. A significant increase was observed up to 26.42857 ± 3.258688 (p≤0.05), up to 33.85714 ± 3.57904 (p≤0.01), up to 34 ± 4.546061 (p≤0.01) at the moment of the blocker addition, during the 7th and the 15th minute of the experiment, respectively. Thus, the blocker has the maximum positive effect on such parameters of the action potential among week-old rats as dpd50% - by 87% at the 15th minute of the experiment and took the value 14.42857 ± 1.98806 (p≤0.01), dpd90% - by 69% during the 7th minute of the experiment which made 22.11486 ± 3.0778342 (p≤0.01), dpd100% - by 36% during the 15th minute of the experiment in comparison with the initial values and took the value of 34 ± 4.546061 (p≤0.01).

During the study of the drug concentration of 10^{-5} M among adult rats, the initial dpd50% value was 8.142857 ± 1.9518. Then a significant increase was observed to 15,12857 ± 5,080917 (p≤0,01) during the 7th minute, and up to 15,28571 ± 6,290583 (p≤0,01) during the 15th minute of the experiment. The initial value of dpd90% was 26.42857 ± 6.528327. When the substance was added, a significant increase was observed up to 29,07143 ± 6,044,478 (p≤0,01), up to 35,14286 ± 6,593648 (p≤0,01) during the 7th minute of the experiment, up to 34,27143 ± 7,513258 (p≤0.01) during the 15th minute of the experiment. The initial value of dpd100% was 41.85714 ± 6.1759. When the substance was added during the 7th and the 15th minute of the experiment they observed a significant increase of the action potential up to 44.92857 ± 6.954786 (p≤0.05), 52.92857 ± 8.358771 (p≤0.05), 50.71429 ± 12.00992 (p≤0.05), respectively. Thus, among 20 week old rats, the maximal positive effect was made by I_f current blocker with the concentration of 10^{-5} M on such parameters of the action potential as dpd50% - by 88% during the 15th minute of the experiment and made 15.28571 ± 6.290583 (p≤0, 01), dpd90% - by 33% during the 7th minute of the experiment and made 35.14286 ± 6.593648 (p≤0.01), dpd100% - by 27% at the 7th minute of the experiment and made 52.92857 ± 8.358771 (p≤0.05) as compared with the original values.

During the study of the same concentration among week-old rats, the initial value of dpd50% was 7.428571 ± 2.935821. When the substance was added during the 7th, and also during the 15th minute of the experiment, we observed a significant increase of this parameter up to 8.785714 ± 3.160395 (p≤0.05), 13.85714 ± 5.014265 (p≤0,01), 15 ± 4.50925 (p≤0.01), respectively. The initial value of the action potential parameter dpd90% among newborn rats was 15.28571 ± 5.1454748. A significant increase was observed at the 7th minute of the experiment to 24 ± 6.855565 (p≤0.01) and to 24 ± 6.806859 (p≤0.01) at the 15th minute of the experiment. The initial value of dpd100% was 28.85714 ± 5.1777791. There was a significant increase to 38,35714 ± 9,463589 (p≤0,01) and to 39,42,857 ± 8,141604 (p≤0,01) during the 7th and the 15th minute, respectively. Thus, ZD7288 in the concentration of 10^{-5} M had the maximum positive effect on such parameters of the action potential as dd50% - by 102% during the 15th minute of the experiment and made 15 ± 4.50925 (p≤0.01), dpd90% - by 57% during the 7th and the 15th minute of the experiment and made 24 ± 6.855565 (p≤0.01) and 24 ± 6.806859 (p≤0.01), respectively, dpd100% - by 37% during the 15th minute of the experiment made 39.42857 ± 8.141604 (p≤0.01) in comparison with the initial values.

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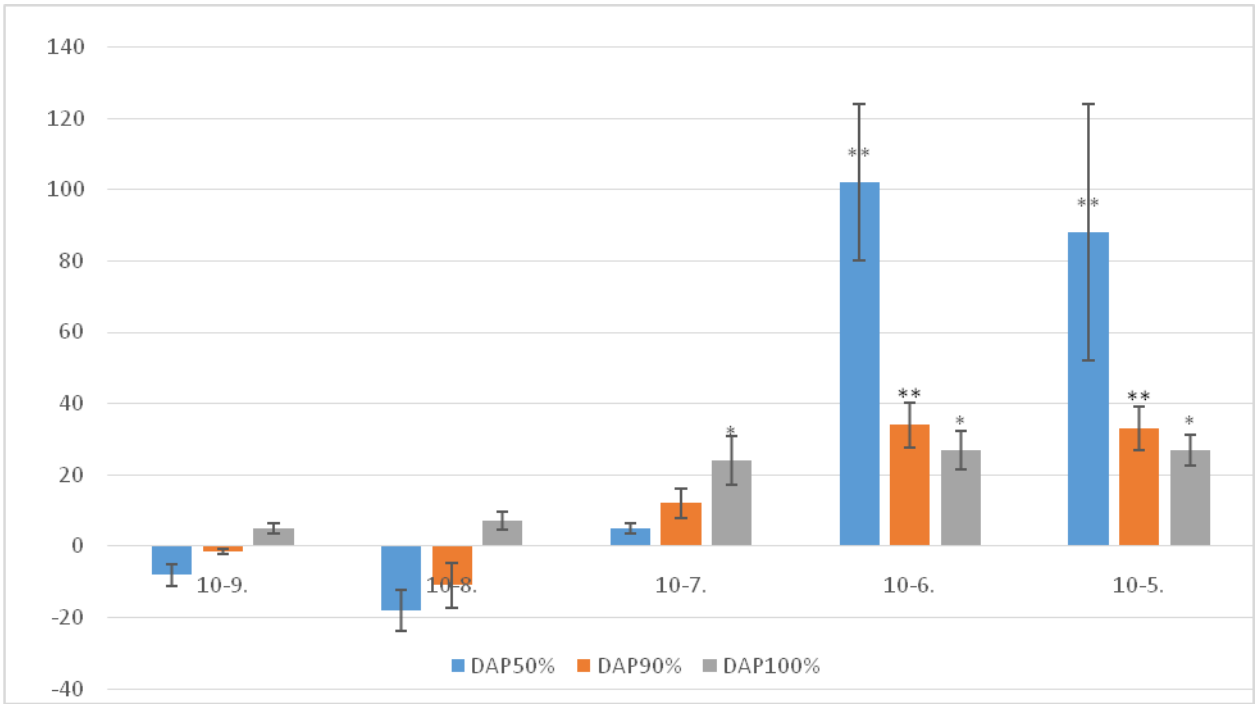

Fig 1: The effect of various concentrations of ZD7288 on the duration of PD at 50%, 90% and 100% repolarization among 20-week-old rats. The ordinate axis is the duration of the action potential (DPD) (%), the abscissa axis is the concentration of ZD7288 (M). Note: the reliability is indicated in comparison with the initial values: * - p<0,05; **- p<0,01.

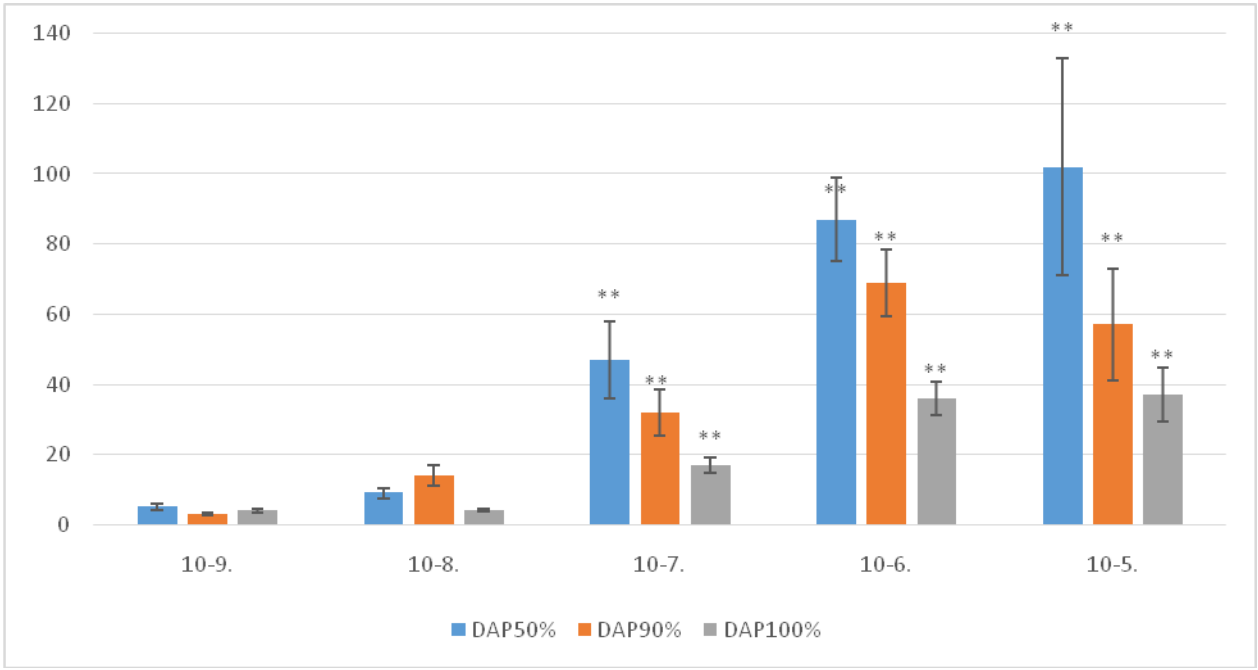

Fig 2: The effect of different concentrations of ZD7288 on the duration of PD at 50%, 90% and 100% repolarization among 1-week-old rats. The ordinate axis is the duration of the action potential (DPD) (%), the abscissa axis are the concentrations of ZD7288 (M). Note: reliability is indicated in comparison with the initial values: **- p<0,01.

CONCLUSIONS

The result of the conducted experiments showed that the blocker of currents activated during hyperpolarization at low concentrations (10-9 M, 10-8 M) does not have significant effects on the duration of the action potential among 1 and 20 week old rats. High concentrations of ZD7288 in both age groups cause a significant increase of the action potential duration at the repolarization level of 50%, 90% and 100%.

SUMMARY

A characteristic feature of atypical myocarditis is the presence of the pacemaker depolarization phase in their action potential, the main role in the formation of which is played by the current activated under hyperpolarization. Since working cardiomyocytes do not produce spontaneous action potentials independently, the role of If-currents in them does not converge with the classical pacemaker function in pacemaker cells. Besides, there was no data on changes in the characteristics of these currents, depending on the stages of ontogeny. The current activated by hyperpolarization was detected in the working myocardium of the ventricles among adult chick embryos [17]. It has also been shown that I_f is present in the physiological range of stress within the ventricular myocardial cells of newborn rats, and in adult animals it may be located depending on the level of the sympathetic innervation of the developing heart maturity and therefore shifts towards negative values [18; 19; 16]. There is information that the intensity of I_f changes with age [7]. It has been proved that the activity of this current in ventricular myocardium cells of old or sick animals is more pronounced with positive membrane potential values [8].

In order to identify the age-specific features of current blockade effect, activated by hyperpolarization on the parameters of the action potential of operational cardiomyocytes, they studied the effect of different concentrations of ZD7288 on the parameters of electrical activity of the working atrial myocardium among newborns and adult rats. Some age-specific differences of ZD7288 effects have been established. For example, among newborn rats, significant changes in PD duration were observed at 50%, 90%, and 100% repolarization in response to ZD7288 at the concentration of 10^{-7} M, whereas among adult rats this concentration of the drug caused DPD increase only at the level of 100% repolarization. Higher concentrations of ZD7288 in both age groups caused a significant increase of the action potential duration at 50%, 90%, and 100% repolarization. However, this increase was more significant among 1-week-old rats. The conducted studies revealed the extension of the repolarization phase in working atrial cardiomyocytes in response to the blockade of If in both age groups. That is, most likely, the activation of If leads to the shortening of PD duration. This case is confirmed by the studies on the simulation of the electrical activity of human ventricular myocardiocytes, which revealed the presence of the output current generated by HCN4 channels during the plateau phase, which leads to PD duration decrease [20]. Thus, HCN channels are the main factors in the repolarization phase of working cardiomyocytes. The fact that HCN channels increase the transmural gradient of repolarization in a healthy heart has potential value for the detection of human heart disease genesis. The existing repolarization gradient among absolutely all types of mammals, including humans, plays a key role in the normal functioning of the heart [15]. It has been established that the increase or the decrease in the duration of the repolarization phase can increase the susceptibility to ventricular arrhythmias [9].

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