Research Article

The role of If and $I_{Ca,L}$ in α-adrenergic regulation of rats cardiac activity

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ABSTRACT

Aim: A heart has a very effective receptor system that interacts with mediators and activates intracellular signaling systems. Adrenoreceptors (AR) are common receptors in a man's body. They are distinguished by a variety of functional responses arising from their activation. The currents activated by hyperpolarization (If) and the potential-dependent L-type Ca^{2+} channels ($I_{Ca,L}$) play an important role in a heart rhythm development. However, their change in ontogenesis can lead to the changes in the work of cardiovascular system. The purpose of this study was to study the effect of α-AR regulation on the chronotropic function of adult rat hearts. **Materials and Method:** A series of *in vivo* experiments was performed on the study of adult rat heart chronotropic function. The right femoral vein was administered with phenylephrine - a non-selective α-AR agonist at the dose of 0.1 mg/kg, ZD 7288 - the preparation blocking If at the dose of 0.07 mg/kg, and verapamil - the blocker of $I_{Ca,L}$ at the dose of 0.1 mg/kg. **Result and Discussion:** The results indicate that the non-selective α-AR phenylephrine agonist causes a significant decrease in the cardiac activity of rats. Phenylephrine against the background of If ZD-7288 blocker causes a significant two-phase decrease in the heart rate. **Conclusion:** The introduction of phenylephrine against the background of $I_{Ca,L}$ verapamil blocker action causes a significant decrease in the cardiac activity of rats.

KEY WORDS: Chronotropy, Currents activated at hyperpolarization, L-type potential-dependent calcium channels, Rat, α-adrenoreceptors

INTRODUCTION

A heart has an effective system of receptors that interact with mediators and modulate the activity of intracellular signaling. It is believed that catecholamines activate β1-adrenoreceptors (AR), β2-AR, and α1-AR most of all. They are located in a myocardium, the blood vessels of the skin, mucous membranes of the brain, the vessels of an abdominal region, kidneys, and an intestine, a spleen, smooth muscles of bronchi,[1] an eye iris, a uterus, and the organs of a genitourinary system.[2] There are 9 subtypes of AR: α1A-, α1B-, α1D-, α2A-, α2B-, α2C-, β1-, β2-, and β3-AP.[3] All ARs are able to modulate the activity of various signaling systems through specific G proteins.[4]

β-AP regulates many functions in a body.[5] In a man's myocardium, α1-AR is less than β-AR, and the amount of α1-AR among rats is 10 times greater.[6] Despite this, α1-AR plays an important role in the regulation of heart functions. The stimulation of α1-AR increases myocardial sensitivity to Ca^{2+} ions, and a prolonged stimulation of cardiac α1-AR can induce rat heart hypertrophy development.[7] It is believed that α2-AR in the heart of mammals performs the modulating functions of regulatory influences. Due to their presynaptic localization, α2-AR inhibits the release of noradrenaline.[8] The presence of α2-AR in a man's heart was demonstrated on the basis of pharmacological analysis and molecular cloning,[9] and α2-AR immunoblotting method was revealed in cardiac tissue of rats.[10] It was shown that the activation of α2-AR causes a positive inotropic effect[11] and a negative chronotropic effect, which is most likely conditioned by the inhibition of norepinephrine release from the sympathetic nerve terminals.[12]

The ability to automatic nature is the most important function of a heart, necessary for a man's life. It is known that the currents activated with hyperpolarization (If) play a major role in the development of diastolic depolarization phase, but there are other incoming ion currents, including Ca^{2+} currents (I_{CaT} and I_{CaL}).[13] Adrenergic stimulation activates If and thus increases

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the heart rate due to β-AR-dependent increase of cAMP level.[14]

The potential-dependent L-type Ca^{2+} channels ($I_{Ca,L}$) participate for the generation of a spontaneous rhythm in atrial myocardium and atypical cardiomyocytes of the conducting system.[15] Ca^{2+} ions participate in the development of the plateau phase of cardiomyocyte potential action. After the inactivation of Ca^{2+} ions transport, the repolarization process is started. Thus, $I_{Ca,L}$ plays a key role in the duration of the refractory period, providing a clear sequence of myocardium electrical activity generation.[16] $I_{Ca,L}$ type is also important in the processes cardiac rhythm disturbance development.[17]

There is evidence in the literature that If and $I_{Ca,L}$ undergo changes in ontogenesis.[18] If currents are present in the cardiomyocytes of the ventricles among adult mammals at extremely negative values of the membrane potential, and in the cardiomyocytes of newborn rat ventricles, If is present in the physiological range of stress. There is evidence that only the intensity of a given current changes and its potential-dependence does not change in time. $I_{Ca,L}$ undergoes age changes. There are the data on the age-related decrease of $I_{Ca,L}$ in ventricles, and about its increase with age and the shift of the potential-dependency toward negative values.[18] Since these currents play an important role in the formation of the heart rhythm, the study of α-AR regulation effect on the chronotropic function of a heart remains relevant.

The purpose of this study was to study the specific features of α-AR regulation mechanisms concerning the chronotropic function of adult rat heart.

METHODS

In vivo experiments were performed on adults (20-week-old ones), white, random bred rats ($n = 20$). The animals were anesthetized with a 25% urethane solution intraperitoneally at 800 mg/1 kg of body weight. Then, the animal was fixed on a special operating table, a fur coat was cut out on the inside surface of the right lower limb, and a skin incision was made, opening the access to the right femoral vein. The right femoral vein was administered with phenylephrine - A non-selective α-AR agonist at the dose of 0.1 mg/kg, ZD 7288 - the drug blocking If at the dose of 0.07 mg/kg, and verapamil - $I_{Ca,L}$ blocker at the dose of 0.1 mg/kg. The introduction of drugs was carried out using insulin syringes, which allow to produce an accurate dosage of injected substances. An electrocardiogram (ECG) was continuously recorded among rats using EC 1T-03M electrocardiograph. The steel electrodes were injected subcutaneously into the limbs of the animals under the study, which allowed to record the signals from the heart steadily. The IInd standard lead was used. The signals came to the oscilloscope C1-83 from the electrocardiograph, and then, to the personal computer where the ECG was processed. The ECG was mathematically processed, and then, 12 values of the variational pulsogram parameters were determined. In our work, we selected 9 parameters reflecting, in our opinion, the activity of the most important mechanisms for the regulation of cardiac activity and for the further statistical processing of a series of animal experiment results such as follows: The average cardio interval value*s* (Xav), the modes (Mo), the amplitude modes (AMO), the variational range (ΔX), the mean square deviation (δ), stress index (SI), vegetative equilibrium index (VEI), vegetative rhythm index (VRI), and the indicator of regulatory process adequacy (IRPA).

The statistical processing of the obtained data and the determination of the result difference reliability concerning the studies by the Student and Wilcoxon test were carried out in the Microsoft Excel program.

RESULTS

A series of experiments with the administration of phenylephrine at the dose of 0.1 mg/kg of the animal's weight was carried out to study the specific effects of a non-selective α-AR agonist introduction on the cardiac activity of rats.

A brief bradycardia was observed with intravenous bolus injection of α-AR phenylephrine agonist. Immediately after the administration of the drug, the Xav value increased from 172.7±11.4 ms to 276.7±26.6 ms ($P < 0.01$) (Figure 1). 1 min after the administration of phenylephrine, Xav exceeded the initial values significantly and made 203.6±3.2 ms ($P \leq 0.05$) (Figure 1). By the 10th min, after the introduction of phenylephrine, the value of Xav was restored to the initial values. The dynamics of heart rate variability indices indicated the increase of parasympathetic channel tone regulation concerning the heart regulation. After the introduction of phenylephrine, the values of Mo and δ increased, the value of ΔX had the tendency to increase. At the same time, the decrease of AMO values was observed from 20.7±4.67% to 5±0.51% ($P \leq 0.05$), VRI decreased from 559.16 ± 144.5 rel. un. to 18.16 ± 4.74 rel. un. ($P \leq 0.05$), the value of IRPA made ($P < 0.05$). 3 min after the administration of phenylephrine, the tendency was registered to SI, IRPA, VRI, and VEI value increase, which indicates the activation of VNS sympathetic department. Perhaps, this is a compensatory response to a significant decrease of the heart work after intravenous phenylephrine introduction.

In the next series of experiments, the study of non-selective α-AR phenylephrine agonist administration

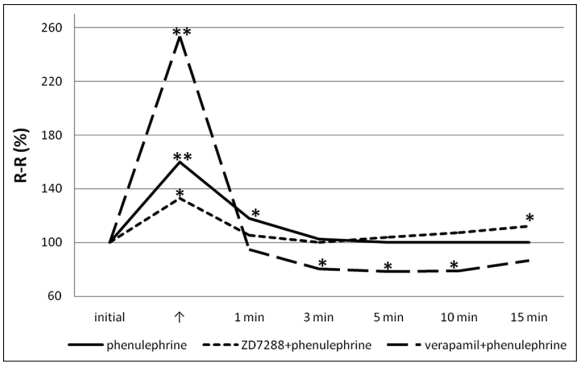

Figure 1: The effect of phenylephrine introduction on the chronotropy of adult rat hearts. The ordinate axis is Xav - an average cardiointerval (R-R, %), the abscissa axis is the recording time of an experiment (minutes). Note: *Reliability in comparison with the initial values: $P < 0.05$; **reliability in comparison with the initial values: $P < 0.01$

effect at the dose of 0.1 mg/kg was performed on the background of If ZD-7288 blocker at the dose of 0.07 mg/kg on the cardiac activity of rats.

Phenylephrine against the background of If blocker action also resulted in a short-term significant increase of the average cardio interval from 195.0 ± 11.9 ms to 259.6 ± 24.7 ms ($P < 0.05$). By the 1st min, after the injection of α-AR agonist against the background of If blockade, the value of Xav was 205.7 ± 12.7 ms, 3 min after - 195.6 ± 10.8 ms. After 15 min of observation, Xav made 30 ± 4.17% ($P \leq 0.05$) from the initial values (Figure 1). Thus, the administration of α-AR agonist before and after the blockade of hyperpolarization-activated cyclic nucleotide-gated (HCN) caused a significant decrease of heart rate, but the bradycardia in response to the administration of phenylephrine after the blockade of If was less pronounced (60% and 32%). At that, the values of Xav were increased 2 times. The first reduction was observed after the administration of phenylephrine, and the second reduction occurred 5 min after the administration of α-AR agonist against the background of HCN blockade. In the dynamics of heart rate variability parameters, there were no two-phase changes in their values. At that, there was the tendency to the decrease of AMO, SI, VEI, and IRPA values and the tendency to the increase of Mo, δ, and ΔX values. The values of VRI decreased ($P \leq 0.05$), all this indicated the increase of the parasympathetic channel tone concerning the regulation of vegetative functions.

In the third series of experiments, the effect of non-selective α-AR phenylephrine agonist administration at the dose of 0.1 mg/kg was studied on the background of the blocker $I_{Ca,L}$ verapamil action at the dose of 0.1 mg/kg.

Phenylephrine against the background of the blocker $I_{Ca,L}$ verapamil led to a short-term and a significant increase of Xav value from 198.85 ± 13.38 ms to 504.3 ± 31.9 ms ($P < 0.01$). By the 1st min, after the injection of phenylephrine, the value of Xav was restored to 188.4 ± 8.2 ms. By the 3rd min after the injection of the α-AP agonist against the $I_{Ca,L}$ blockade, the Xav value decreased to 159.6 ± 4.8 ms ($P < 0.05$), this decrease was maintained up to the 10th min of observation ($P < 0.05$) (Рис.1). By the 15th min, after the agonist injection, the value of Xav was smoothly restored. At that, the decrease of VEI ($P = 0.05$), SI ($P = 0.05$), and VRI ($P = 0.01$) values and the increase of δ ($P < 0.05$), Mo ($P < 0.05$), and δX ($P < 0.01$) were observed. These changes indicated the decrease in the tone of the sympathetic regulation channel. Thus, the reduction after an intravenous administration of phenylephrine against verapamil was a short one, but more pronounced, as compared with the results after the administration of pure phenylephrine (155% and 60%). Moreover, the values of Xav increased similarly to the results with the introduction of pure phenylephrine immediately after the administration, and at the 3rd min after the injection of α-AR agonist, there was the increase in heart rate against the background of verapamil effect.

CONCLUSIONS

The introduction of a non-selective α-AR phenylephrine agonist causes a significant decrease of rat cardiac activity. The introduction of phenylephrine against the background of the blocker If ZD-7288 causes a significant two-phase decrease in the heart rate and reduces the severity of bradycardia. The introduction of phenylephrine against the background of the verapamil blocker $I_{Ca,L}$ causes a more pronounced decrease in the cardiac activity of rats.

SUMMARY

Based on the obtained results of the conducted experiments, it can be concluded that the introduction of a non-selective α-AR phenylephrine agonist caused a significant decrease in the cardiac activity of 20-week-old rats, which made 60% ($P < 0.01$). The variations in the parameters of the variation pulsogram were compensatory ones. After the introduction of phenylephrine, their dynamics indicated the increase of parasympathetic regulation level concerning cardiac activity. Then, when the heart rate was restored, the dynamics of the variational pulsogram parameters indicated the increase in the tone of the channel sympathetic regulation.

The introduction of phenylephrine against the background of If blockade led to a significant decrease in the heart rate, as well as with the introduction of pure phenylephrine. In this case, the decrease in response to the introduction of phenylephrine against the background of the blocker If was less pronounced

(60% and 32%). However, in the dynamics of the values of Xav, a two-phase decrease in the heart rate was observed. In the dynamics of the variational pulsogram parameter values of two-phase changes, their values were not observed. After the introduction of phenylephrine against the background of If blockade, the increase in the tone of the autonomic nervous system parasympathetic part was observed.

The reduction in response to the introduction of phenylephrine against the background of the blocker $I_{Ca,L}$ was very short, but more pronounced one, as compared with the effect of pure phenylephrine (155% and 60%). Moreover, the dynamics of Xav values showed a two-phase change in the heart rate. First of all, just after the administration, the decrease was observed similar to the series with pure phenylephrine. Then, from the 3rd min, after the administration of phenylephrine against the background of the verapamil blocker action, there was the increase in the work of the heart. The dynamics of the variational pulsogram parameters did not show two-phase changes, only the increase in the tone of the parasympathetic regulation channel was observed simultaneously with the decrease in the work of heart. The I_{CaL} blocker verapamil did not eliminate the bradycardic effect of phenylephrine. At the same time, the subsequent dynamics of heart rate variability indices changed.

Thus, the series of experiments on the identification of If and $I_{Ca,L}$ role in the mechanisms of adrenergic regulation of the heart chronotropic function showed that the blockade of If and $I_{Ca,L}$ did not abolish the bradycardic effects of phenylephrine. However, the change in the severity of bradycardia indicated the modulating effect of these currents in the regulation of cardiac activity from α-adrenergic receptors.

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