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Role of RyR2 and SERCA2a in the Cardioprotective Effects of Vincanine and Pyrazoline Alkaloids

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Abstract

In the conducted studies, the effect of vincanine and pyrazoline indole alkaloids on cardiac muscle cell sarcoplasmic reticulum **(**SR) Ca^{2+}-transport systems (RyR2 and SERCA2a) was investigated under normal and hypoxic conditions. The effect of vincanine and pyrazoline on ryanodine receptor (RyR2) was evaluated in the presence of ruthenium red and it was found that the role of RyR2 in the positive inotropic effect of these alkaloids is small. Also, in the presence of cyclopiazonic acid (IC_{50} = 5.6 μM), an inhibitor of sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2a), the positive inotropic effect of vincanine and pyrazoline alkaloids was reduced. In general, the positive inotropic effects and cardioprotective properties of vincanine and pyrazoline alkaloids are mediated by modulation of SERCA2a function.

Keywords: Indole alkaloid, Papillary muscle, Inotropic activity, Sarcoplasmic reticulum, Ion-channels

Introduction

The development of the majority of cardiovascular diseases, the etiology of which is not different [1], is mainly related to the disturbance of the intracellular circulation of Ca^{2+}, which plays a leading role in the regulation of the functional and secretory activity of the myocardium [2]. Circulation of Ca^{2+} in cardiomyocytes is ensured by the Ca^{2+}-transporting system, where the potential-dependent Ca^{2+}-channel L-type plays a key role, the activation of which in the development of action potential is accompanied by the promotion of Ca^{2+} in cardiomyocytes [3-5]. The incoming Ca^{2+} ions stimulate the massive release of Ca^{2+} from the SR through the RyR2, contributing to a tenfold increase in the intracellular concentration of Ca^{2+} and the initiation of the process of myocardial contraction [6,7]. Relaxation of the myocardium, after the act of contraction, occurs as a result of restoration of the initial level of Ca^{2+}, which is mainly ensured by their pumping into the SR by specialized SERCA2a, and partially by their removal from the Na^{+}/Ca^{2+} exchanger [8,9]. The dynamic fluctuation of the level of Ca^{2+} ions in cardiomyocytes ensures the rhythmic alternation of the processes of contraction and relaxation of the heart muscle, which is necessary for the implementation of the heart's normal pumping fuction. It was found that disturbances in the circulation of Ca^{2+} in cardiomyocytes, observed in cardiovascular diseases, occur mainly at the level of SR, the main depot and regulator of myocardial contractile activity [10]. It has been established that these disorders are based on damage to the mechanisms regulating the activity of RyR2 and SERCA2a, which lead to uncontrolled leakage of Ca^{2+} from the SR, depletion of their reserves in the latter and overload of cardiomyocytes with Ca^{2+} ions [11,12]. The overload of cardiomyocytes with Ca^{2+} ions is a key trigger for various pathological processes

that cause serious damage, not only to cardiomyocyte electrical and contractile activity but also to their structural organization.

In addition, in cardiovascular diseases, excessive stimulation of β-adrenergic receptors and increased activity of PKA and CaKMPKII promote hyperphosphorylation of RyR2 and disruption of its association with calstabin 2, which leads to uncontrolled leakage of Ca^{2+} ions from the SR and a decrease in their content in the latter [13-15]. At the same time, the pumping of Ca^{2+} into the SR is significantly reduced, which is provided by SERCA2a, the activity of which is controlled by the regulatory protein phospholamban (PLN) [16,17]. Normally, phosphorylation of phospholamban PKA and CaKMPKII activates Ca^{2+}-ATPase and the process of pumping Ca^{2+} ions into the SR [18]. However, it has been established that in cardiovascular diseases there is a noticeable decrease in the activity of Ca^{2+}-ATPase, due to impaired phosphorylation of PLN and a decrease in the expression of the pump. It is quite obvious that defects in the regulation of RyR2 and Ca^{2+}-ATPase, which lead to critical accumulation of Ca^{2+} in cardiomyocytes, are the main cause of the development of functional and structural disorders of the myocardium [19,20]. In this regard, there are protective mechanisms in the myocardium, which are mainly aimed at limiting the excessive accumulation of Ca^{2+} in cardiomyocytes.

Indole alkaloid rhynchophylline isolated from the plant *Uncariarhynchophylla*, has a pronounced inotropic effect and modulates Ca^{2+} transport in cardiomyocytes by modifying the processes of Ca^{2+}pumping or release into the SR [21].Vincanine alkaloid isolated from *Vinca erecta* has also been found to have a positive inotropic effect on cardiac muscle contractile activity [22]. In view of the above, in our research, the effects of vincanine and pyrazoline indole alkaloids on cardiac muscle cells (SR RyR2 and SERCA2a) were investigated.

Material and methods

Registration of contractile activity of the papillary muscle of the rat heart

The studies were carried out on preparations of papillary muscle isolated from the right ventricle of outbred white rats, weighing 200 - 250 g. The animals were immobilized with light ether anesthesia, decapitated, and after opening the chest cavity, the heart was removed and placed in a physiological Krebs solution of the following composition (in mM): NaCl – 120; KCl – 4.8; $CaCl_2$ – 2; $MgSO_4$ – 1.2; KH_2PO_4 – 1.2; $NaHCO_3$ – 20; glucose – 10, pH = 7.4 [23]. The work used preparations of papillary muscle isolated from the right ventricle of the heart with an average diameter of 0.5 - 0.8 mm and a length of 1 - 3 mm. The contraction activity of papillary muscle preparation was recorded using a mechanographic device (Mayflower Tissue Bath System, Hugo Sachs Electronic, Germany) and a hardware-software complex (LabScibe 2, World Precision Instruments, USA). The experimental chamber is perfused with oxygenated (O_2–95%, CO_2–5%) Krebs solution at a rate of 6 mL/min [24,25]. After the stabilization period, the muscle was stimulated with rectangular pulses with a frequency of 0.1 Hz and a duration of 5 ms and an amplitude exceeding the threshold by 20 %, using an ESL-2 electrical stimulator. After a stabilization period, the length of the preparation was found at which the muscle develops maximum isometric tension (L_{max}) and all experiments were performed under these conditions.

Hypoxia model *In vitro*

To evaluate the potential cardioprotective activity of the compounds, a hypoxia model was used, which was obtained by replacing oxygen in the Krebs perfusion solution with nitrogen (N_2). This model was created by incubating segments of rat heart papillary muscle in Krebs solution, aerated with a mixture (95% N_2/ 5 % CO_2) [26,27]. The effect of hypoxia and assessment of the potential cardioprotective activity of the compounds was carried out after a one-hour replacement of oxygen in the perfusion solution with nitrogen, under conditions when maximum changes in the parameters of the contractile activity of rat papillary muscle preparations occurred.

The development of hypoxia in the heart muscle is accompanied by a significant decrease in contraction force, which is mainly due to dysfunction of Ca^{2+} transport systems and Ca^{2+} homeostasis in cardiomyocytes [28]. In this case, the key role is played by damage to the functions of RyR2 and Ca^{2+}-ATPase SR, which play a leading role in maintaining Ca^{2+} homeostasis in cardiomyocytes [29]. In our experiments, perfusion of the papillary muscle of the rat

heart with a hypoxic solution for 60 min led to a decrease in the amplitude of contractions by 74.6±5.2 % (**Figure 1**).

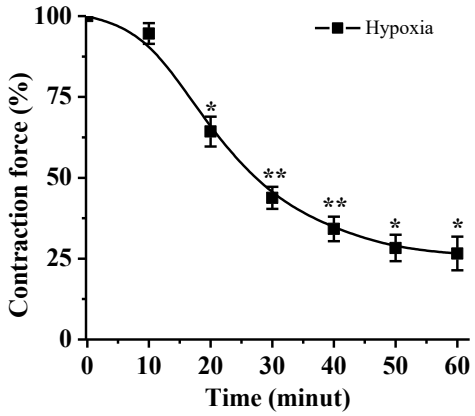

Figure 1 Effect of hypoxia on the contractile activity of the papillary muscle of the rat heart. On the ordinate axis - muscle contraction force, the control obtained under normal oxygenation of the physiological solution is expressed as a percentage and taken as 100 %. On the abscissa axis is the deoxygenation time of the solution perfused with nitrogen. The drug was stimulated with a frequency of 1 Hz. * – $p < 0.05$, ** –$p < 0.01$ (n = 5).

Chemicals

The following pharmacological preparations and reagents were used in the work: ruthenium red, cyclopiazone acid, NaCl, KCl, $NaHCO_3$, NaH_2PO_4, $CaCl_2$, and $MgSO_4$ (Sigma, USA). The alkaloids vincanine and pyrazoline have been isolated a Vincanine from the roots of Vinca erecta (fam. Apocynaceae) at the Institute of the Chemistry of Plant Substances of Academy of Sciences of Uzbekistan and ware kindly provided.

Statistics

Throughout this article, all data are expressed as mean ±SD. Control values between groups were compared by analysis of variance. The Student's *t*-test was used to compare two means. A probability of less than 0.05 was taken as a statistically significant difference. Statistical analysis was performed using OriginPro 9.1 software (OriginLab Co., U.S.A).

Results and discussion

In the experiments, we investigated the effect of pyrazoline and vincanine alkaloids on myocardial cell SR Ca^{2+}-transport systems. Studied the effect of alkaloids on SR Ca^{2+}-transport systems was researched during post-rest potentiation. The process of post-rest potentiation is explained by the sudden increase in the initial contraction force when cardiac muscle stimulation is stopped for 30 s and resumed after a certain period of rest.After resting for 30 s, cardiomyocytes accumulate more Ca^{2+} ions in the SR compared to their previous physiological state, and upon restimulation, more Ca^{2+} ions are released into the cytosol [30]. In this case, a sharp increase in the initial contraction force is observed.Post-rest potentiation is a widely used adequate method for studying changes in $[Ca^{2+}]_{SR}$ concentration in cardiomyocytes [31]. As a result of the contraction of cardiomyocyte cells, due to the release of Ca^{2+} ions from the SR, cardiomyocytes take in more Ca^{2+} ions than in the physiological state and and cause more Ca^{2+} ions to be released after recovery of excitation [32].

Previous studies have shown that vincanine and pyrazoline alkaloids have positive inotropic effects when examining the dose-dependent effects of vincanine and pyrazoline alkaloids on the contractile activity of rat cardiac papillary muscle. It was observed that vincanine 50 μM and pyrazoline 70 μM increased the force of papillary muscle contraction of rat heart by 73.9±3.1 and 45.4±3.7 % compared to the control [22].

In our control experiments, it was found that when papillary muscle stimulation was interrupted for 30 s

and then returned to the previous stimulation, the amplitude of the first contraction force increased by 81.4±5.6 %. Under these conditions, we investigated the effect of vincanine (50 μM) and pyrazoline (70 μM) alkaloids on the value of post-rest potentiation. It was found to increase the amplitude of the first contraction after the rest period by 25.2±3.6% and 32.8±4.4 % in comparison to the control (**Figure 2**).

According to the analysis of the results of this experiment, it was found that the effect of vincanine on SR function is relatively stronger than that of pyrazoline. In this case, these alkaloids increase the amount of Ca^{2+} ions released from the SR, and as a result, the value of PRP increases. The positive inotropic effect of the studied biologically active substances is partly explained by the effect on increasing the amount of Ca^{2+} ions collected and released in the SR.

In subsequent experiments, we investigated the effect of these alkaloids on RyR2, using the inhibitor of RyR2, ruthenium red. In the presence of ruthenium red (15 μM), the post-rest potentiation value of vincanine and pyrazoline alkaloids was found to decrease by 18.6±4.8% and 27.4±3.9%, respectively, compared to the control (**Figure 3**).

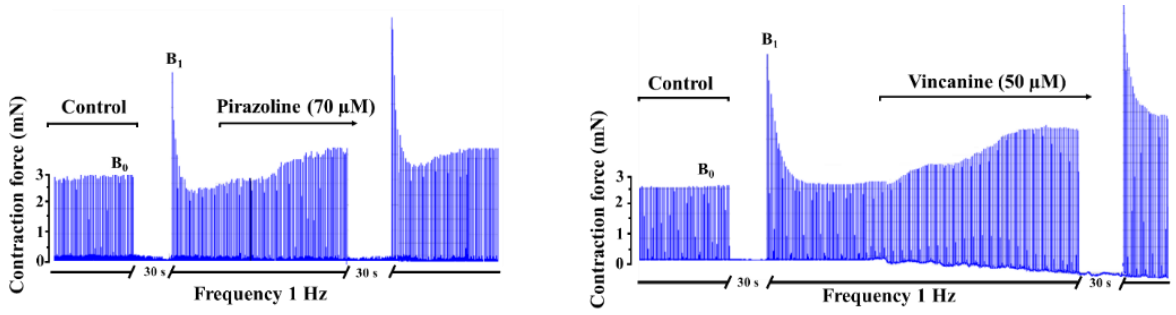

Figure 2 Effects of pyrazoline and vincanine alkaloids on papillary muscle post-rest potentiation value. Stimulation stop time is 30 s. The contraction force of the muscle after rest is taken (post-rest potentiation) as 100 %. Increase in post-rest potentiation value under the influence of pyrazoline and vincanine alkaloids. The frequency of stimulation of the drug is 1 Hz (n = 4).

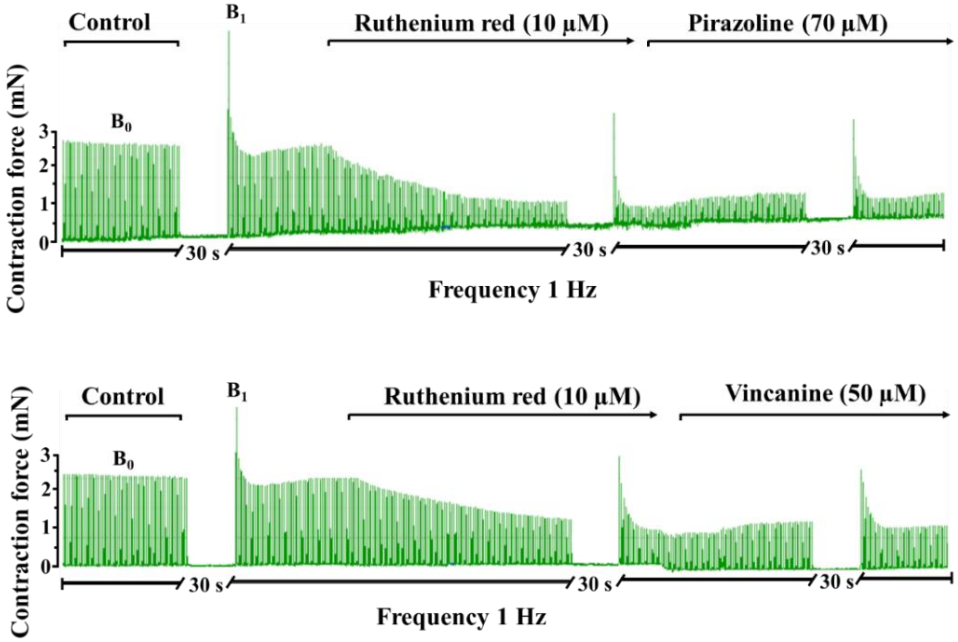

Figure 3 Effect of pyrazoline and vincanine alkaloids on papillary muscle post-rest potentiation value in the presence of ruthenium red. Stimulation stop time is 30 s. The control contraction force of the muscle after rest (post-rest potentiation) was taken as 100 %. The frequency of stimulation of the drug is 1 Hz (n = 4).

As an additional proof of these studies, in the next experiments, the effect of vincanine and pyrazoline alkaloids on the dynamics of SR Ca^{2+} was analyzed based on the effect on the amplitude of the single contraction force that occurs under the conditions of rapid cooling of the incubation medium (+37⟶+4 °C). Under the influence of rapid-cooling contracture (RCC) of the myocardial drug incubation medium, the sarcolemma is depolarized and an action potential is generated. In turn, due to the activation of RyR2 under the influence of Ca^{2+} ions entering the cytosol, the concentration of Ca^{2+} ions in the cytosol increases, and the amplitude value of the RCC single contraction that occurs is used as an index of the dynamics of $[Ca^{2+}]_{CP}$ ⟶ $[Ca^{2+}]_{in}$↑ [33,34]. Normally, during RCC, $Ca^{2+}]_{in}$ is regulated by NCX1, therefore, under $[Na^{+}]_{out}$=0 conditions, i.e. when the function of NCX1 to transport Ca^{2+} ions out of the cell is blocked, RCC amplitude value increases [35].

In the experiments, it was found that in the presence of vincanine and pyrazoline alkaloids, the contraction force of the developing muscle under rapid cooling conditions increased by 31.7±5.1 and 38.6±3.9 % compared to the control (**Figures 4(A)** and **4(B)**).

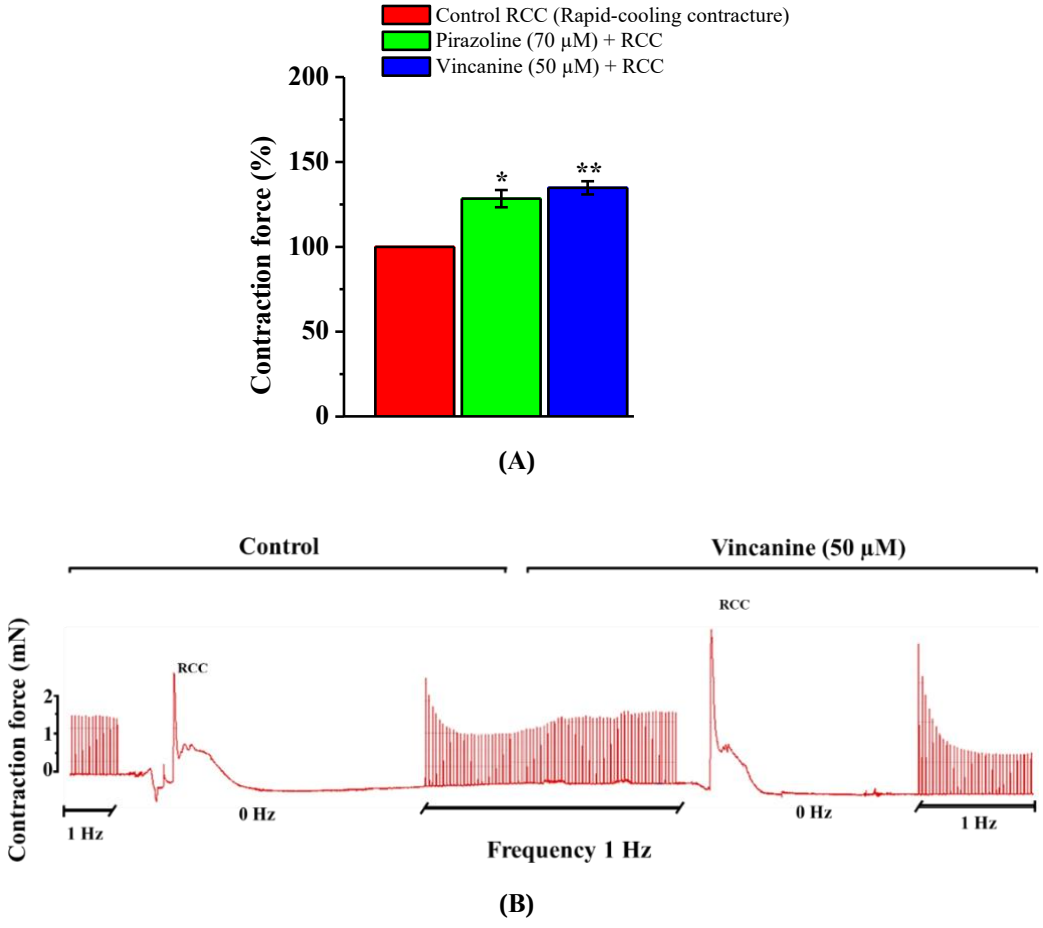

Figure 4 (A) Effects of vincanine and pyrazoline alkaloids on papillary muscle contraction induced by rapid cooling of myocardial preparation. The temperature gradient is from +37 to +4 °C. On the ordinate axis is the contraction force of the papillary muscle expressed as a percentage of the RCC value taken as 100 %. * – $p < 0.05$, ** –$p < 0.01$ ($n = 4$). (B) An increase in the amplitude of contractile force (RCC) under the influence of vincanine during rapid cooling (+37⟶ +4 °C) of the incubation medium of the myocardial preparation (*original record*).

RCC is associated with an increase $[Ca^{2+}]_{in}$ via RyR2 activation in cardiomyocytes, and with this method, changes in myofilament Ca^{2+}-sensitivity can be analyzed together with the dynamics of $[Ca^{2+}]_{SR}$ change. Myocardial preparation is associated with an increase in "peak" myofilament Ca^{2+}-sensitivity in the form of a rapid increase in contraction force under conditions of reheating of the incubation medium (+4⟶+37 °C) [36]. Based on the results of the above research, it can be said that the positive inotropic effect of vincanine and pyrazoline alkaloids on the papillary muscle contraction activity of the rat heart indicates the partial involvement of RyR2, which is important in the release of Ca^{2+} ions from SR to the cytosol and Ca^{2+} homeostasis.

Evaluation of the involvement of SERCA2a in the positive inotropic effects of vincanine and pyrazoline

SR Ca^{2+}-ATPase (SERCA2a) plays an important role in the regulation of Ca^{2+} ion concentration and Ca^{2+}-homeostasis in cardiomyocytes ensures the entry of Ca^{2+} ions into the SR and plays a key role in cardiac muscle relaxation [37]. Based on the results of the above experiments, the positive inotropic effect of vincanine and pyrazoline indole alkaloids may be related to the modification of the processes of accumulation of Ca^{2+} ions in the SR. To clarify this assumption, we investigated the effects of the studied biologically active substances on SERCA2a. Experiments were conducted with the participation of SERCA2a inhibitor - cyclopiazonic acid (CPA) [38,39].

In control experiments, the effect of CPA on papillary muscle contractile activity in a dose-dependent manner (1 - 10 μM) at a stimulation frequency of 1 Hz was investigated. A high concentration of 10 μM of CPA was found to reduce the force of papillary muscle contraction by 80.7±4.8 %. The half-maximal inhibitory concentration of CPA was IC_{50}-5.6 μM (**Figure 5**). Therefore, CPA inhibits SERCA2a and reduces the force of papillary muscle contraction.

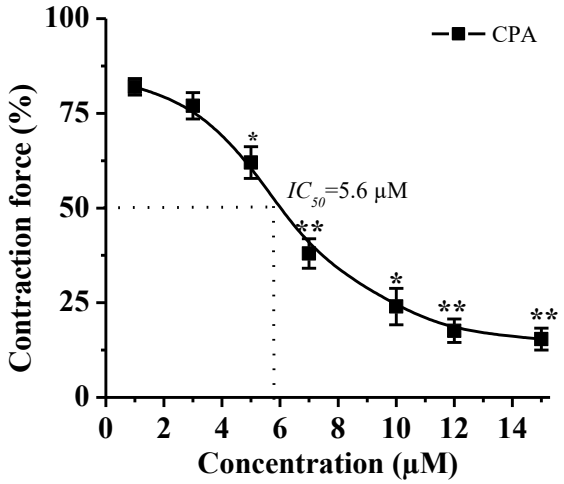

Figure 5 Dose-dependent effect of CPA on papillary muscle contractile activity. On the ordinate axis - the amplitude value of the contraction force expressed as a percentage (%) compared to the maximum, on the abscissa axis - the concentration of CPA (μM) is shown (*−$p < 0.05$; **−$p < 0.01$). Frequency of stimulation 1 Hz, t = 36°C; n = 4.

In subsequent experiments, changes in papillary muscle contraction force under the influence of indole alkaloids vincanine (50 μM) and pyrazoline (70 μM) were investigated in the presence of the half-maximal inhibitory concentration (IC_{50}-5,6 μM) of the SERCA2a inhibitor CPA. In this case, it was found that in the presence of CPA (IC50-5.6 μM), vincanine and pyrazoline alkaloids increase the muscle contraction force by 21.4±3.8 and 14.7±4.4 %, respectively, compared to the control (**Figure 6**).

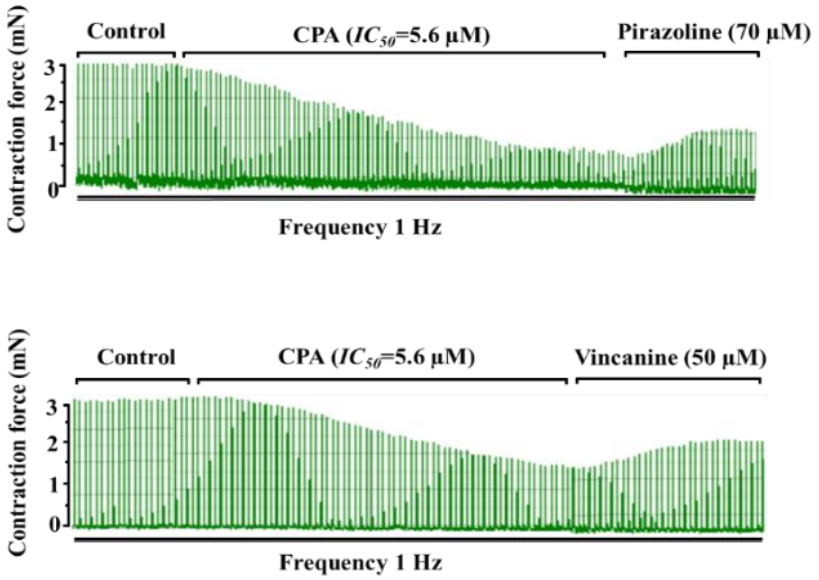

Figure 6 Effects of vincanine and pyrazoline indole alkaloids on papillary muscle contraction force in the presence of CPA (IC_{50}-5.6 μM) in the incubation medium.

These experimental results indicate the involvement of SERCA2a in the positive inotropic effects of alkaloids. The positive inotropic effect of the studied alkaloids on papillary muscle contraction activity is explained by the fact that SR Ca^{2+}-transport systems play an important role and that RyR2 is less involved and mainly has a stronger effect on SERCA2a. It also suggests that the potentiating effect of these alkaloids is related to the process that ensures the accumulation of Ca^{2+} ions in the SR.

Effects of vincanine and pyrazoline on SERCA2a under hypoxic conditions

Considering that the force of papillary muscle contraction is carried out with the participation of Ca^{2+} ions, the results of these experiments show that the decrease in muscle contraction force under hypoxia is accompanied by a decrease in $[Ca^{2+}]_{in}$ ions [40].

When studying the effect of vincanine (50 μM) and pyrazoline (70 μM) alkaloids on papillary muscle contraction force under hypoxia conditions, it was found that they restore the papillary muscle contraction activity disruption caused by hypoxia to 88.8±4.3 and 69.6±5.2 % (**Figure 7**).

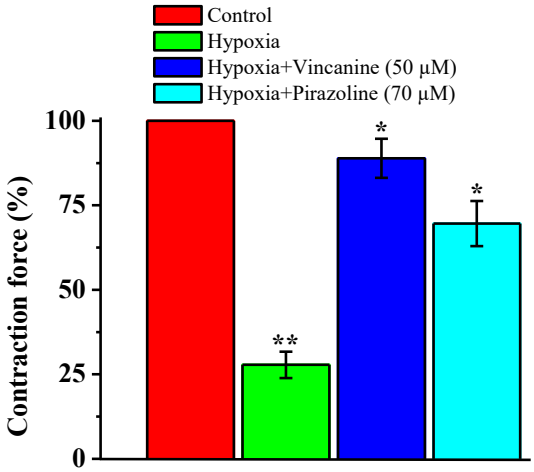

Figure 7 Effects of vincanine and pyrazoline alkaloids under hypoxic conditions on papillary muscle contractile activity of rat heart. The ordinate axis shows the amplitude value of the contraction force expressed as a percentage (%) compared to the maximum. Frequency of stimulation 1 Hz, * $-p < 0.05$; ** $-p < 0.01$; n=5.

Also, in subsequent experiments, the effects of the studied alkaloids on myocardial cell SERCA2a under hypoxic conditions were investigated. Experiments were continued in the *in vitro* hypoxia model. According to the results of the ban, during 60 min of aeration, the force of papillary muscle contraction was found to decrease to 27.6±3.1% compared to the control. Under these conditions, when the effect of vincanine (50 μM) and pyrazoline (70 μM) indole alkaloids was examined in the presence of CPK (IC_{50}-5.6 μM), the force of papillary muscle contraction compared to the control (the percentage of the amplitude of the force of papillary muscle contraction caused by control hypoxia was 19.2 %) were 29±4.2 and 21±4.8 %, respectively (**Figure 8**).

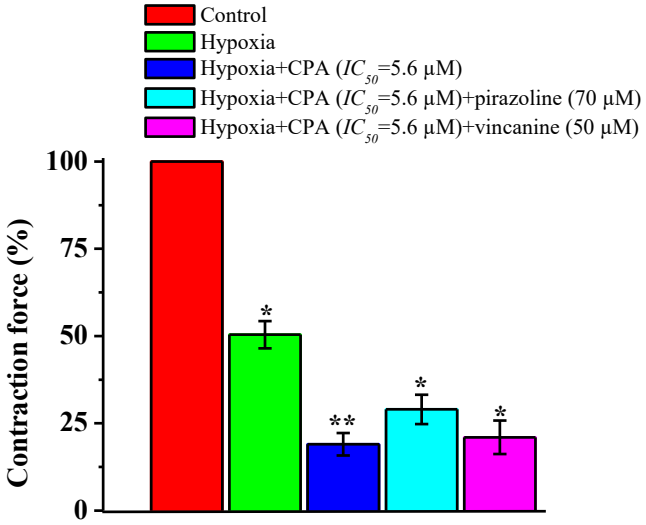

Figure 8 Effects of vincanine and pyrazoline indole alkaloids on papillary muscle contraction force in the presence of cyclopiazonic acid (IC_{50}-5.6 μM) under hypoxic conditions. On the ordinate axis, the force of papillary muscle contraction is expressed as a percentage (%). Frequency of stimulation 1 Hz, t = +36±0.5 °C; n = 4.

From the analysis of the obtained results, it can be concluded that the positive inotropic effect on papillary muscle contraction activity, as well as the increase in the amount of Ca^{2+} ions in the cytosol, is related to the function of the SERCA2a system.

Conclusions

Vincanine and pyrazoline alkaloids effectively reverse hypoxia-induced SERCA2a dysfunction and normalize changes in rat cardiac papillary muscle contractile activity. This can be done through special signal systems that provide communication between SR, control the concentration of intracellular Ca^{2+} ions and SR function. The obtained results showed that the studied biologically active substances have a positive inotropic effect on the contraction activity of the papillary muscle of the rat heart, and the participation in the activation of RyR2 in cardiomyocytes is less, which is mainly characterized by an increase in the activity of SERCA2a.

Acknowledgments

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