

Mechanism of Positive Inotropic Effect of Vincanine on Cardiac Muscle Contraction Activity

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In the present study, the dose-dependent effect of the alkaloid vincanine on the contractile activity of rat heart papillary muscle was investigated and it was found to increase the force of muscle contraction at all concentrations. The half-maximal inotropic effect concentration (ED50) of vincanine was 21.8 μM , respectively. Studies of the effect of this alkaloid on the cardiomyocyte Na^+ -channel in the presence of its blocker lidocaine showed that the positive inotropic effect of vincanine does not involve the Na^+ -channel. When studying the effect of vincanine on α_2 -L-channels in the presence of nifedipine ($\text{IC}_{50}=0.01 \mu\text{M}$), the positive inotropic effect of vincanine (50 μM) alkaloid was partially reduced. In general, it was found that the positive inotropic effect of vincanine on the force of papillary muscle contraction is partially mediated by Ca^{2+} -L-channels and mainly $\text{Na}^+/\text{Ca}^{2+}$ exchange.

Keywords: Cardiac Muscle; Inotropic Activity; Ion-Channels; Indole Alkaloid.

It is known that several cardiovascular diseases are directly related to defects and disturbances in the work of various ion channels in heart cells¹⁻³. The rhythmic contraction of the heart takes place depending on Ca^{2+} ions and is determined by the function of ion channels and receptors located in the cardiomyocyte sarcolemma and SR membrane^{4,5}. During the action potential (AP) in cardiomyocytes, the contraction process begins as a result of the activation of the SR Ca^{2+} -channel of the Ca^{2+} ions entering through the L-type Ca^{2+} -channel located in the sarcolemma, and the Ca^{2+} ions leaving the SR binding to troponin-C in

the myofilament, and during relaxation, of $[\text{Ca}^{2+}]_i$ in the cytosol in the sarcolemma located $\text{Na}^+/\text{Ca}^{2+}$ -exchanger and is normalized by the function of SR Ca^{2+} -ATPase⁶. It has been noted that disturbances in $[\text{Ca}^{2+}]_i$ dynamics in cardiomyocytes⁷, as well as changes in the normal function of SR Ca^{2+} -ATPase⁸, RyR⁹, and $\text{Na}^+/\text{Ca}^{2+}$ exchanger¹⁰. Special attention is paid to the biologically active compounds extracted from the plant, which have been used in folk medicine for centuries to treat various diseases of the cardiovascular system. Examples of this include alkaloids, flavonoids, and other biologically active substances¹¹⁻¹⁴. In

particular, indole alkaloids are important as a promising source for the creation and production of new cardioprotective agents, distinguished by their diverse structures and wide pharmacological effects. Therefore, the study of the properties of the biological effects of these alkaloids is the basis for identifying potential candidates for the creation of a new generation of cardioprotective agents in the treatment and prevention of ischemic heart diseases. Taking this into account, in our research, the mechanism of the inotropic effect of vincanine on the force of papillary muscle contraction of the rat heart was investigated.

MATERIAL AND METHODS

The studies were carried out on papillary muscle preparations isolated from the right ventricle of outbred white rats weighing 200-250 g. Animals were immobilized under light ether anesthesia, decapitated, after opening the chest

cavity, the heart was removed and placed in Krebs physiological solution of the following composition (in mM): NaCl, 120; KCl - 4.8; CaCl₂ - 2; MgSO₄ - 1.2; KH₂PO₄ - 1.2; NaHCO₃ - 20; glucose - 10, pH = 7.4.). We used papillary muscle preparations 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 muscle preparation was fixed with silk threads in the experimental chamber, and one end was connected to the rod of an F30 isometric sensor (Hugo Satche, Germany). The experimental chamber was perfused with oxygenated (O₂ - 95%, CO₂ - 5%) Krebs solution at a rate of 6 ml/min. After the stabilization period, the muscle was irritated 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. The amplitudes of elicited maximal isometric contractions were used as the control (100%) and changes in the contractile force after drug action was expressed as a percentage of

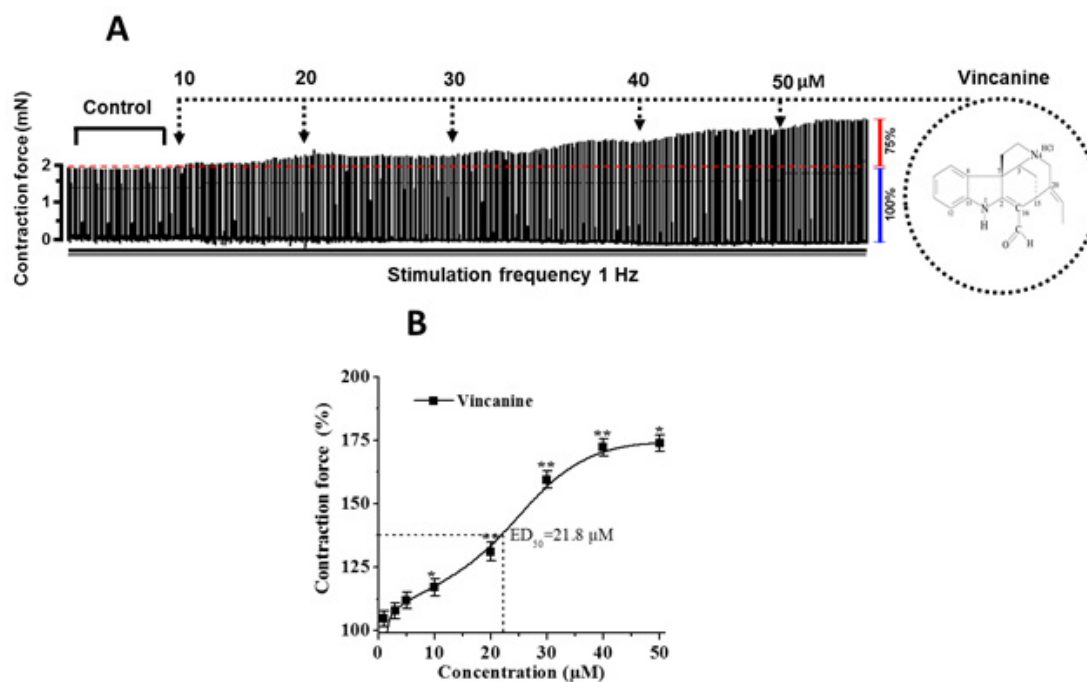


Fig. 1. Effect of vincanine alkaloid on the contractile activity of the papillary muscle of the rat heart

A. Increasing the force of contraction of the papillary muscle of the alkaloid vincanine (original recording). **B.** Dose-dependent increase in the force of contraction of the papillary muscle of the alkaloid vincanine. The y-axis shows the force of contraction of the papillary muscle, expressed as a percentage of the control, taken as 100 %, the abscissa shows the alkaloid concentration (μM), (*- $p < 0.05$; **- $p < 0.01$). Stimulation frequency 1 Hz ($t = +36 \pm 0.5$ °C; $n = 5$)

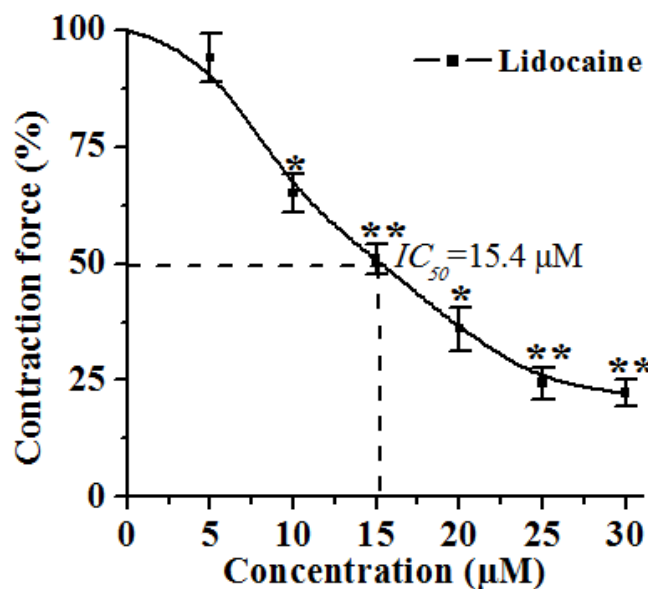


Fig. 2. Dose-dependent effect of lidocaine on papillary muscle contractile activity. On the axis of the ordinate - the amplitude value of the contraction force is expressed as a percentage (%) compared to the maximum, on the axis of the abscissa - the concentration of lidocaine (μM) is shown, (*-p<0.05; **-p<0.01). Stimulation frequency 1 Hz (t=+36±0.5 °C); n=5.

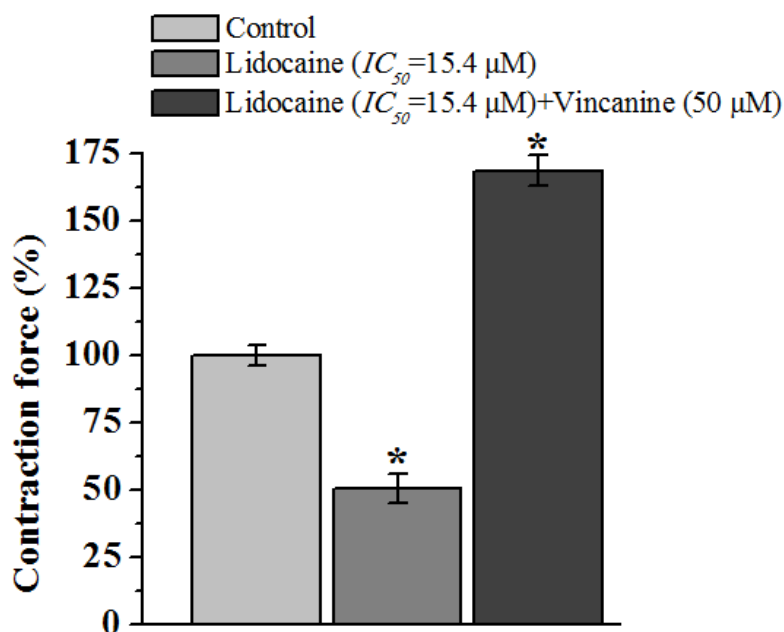


Fig. 3. Effects of vincaniline on papillary muscle contraction force in the presence of lidocaine. On the ordinate axis - the amplitude value of the contraction force is expressed as a percentage (%) compared to the maximum. *-p<0.05 in all cases; n=5.

the maximal response. Contractions were recorded on a chart recorder (TZ 4620, Czech Rep.) and after conversion to digital form stored on a personal computer. Lidocaine was used in the experiments to investigate the effect of Vincanine on the Na^+ -channel, nifedipine was used on the $\tilde{\text{Na}}^{2+}$ channel, and NiCl_2 was used to study the effect of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger function

Drugs and Reagents

Vincanine indole alkaloid was isolated from *Vinca erecta* plant by Adizov M. Shakhobiddin senior researcher (Ph.D.), at the Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan. Lidocaine, Nifedipine, and nickel chloride were purchased from Sigma-Aldrich Chimie (Sigma, St. Louis, MO, U.S.A.).

RESULTS AND DISCUSSION

In preliminary experiments, the dose-dependent effect of vincanine alkaloid on the contractile activity of the rat heart papillary muscle was studied. It was found that this alkaloid has a

positive inotropic effect on the contraction activity of the papillary muscle of the rat heart at all concentrations (Fig.1 A). When vincanine alkaloid was examined at a concentration of 5 μM to 50 μM , it was observed that this alkaloid showed a maximum effect at a concentration of 50 μM and increased muscle contraction force by $73.9 \pm 3.1\%$ compared to the control (control was taken as 100%). The half-maximal inotropic effect concentration (ED_{50}) of vincanine was 21.8 μM , respectively (Fig.1 B).

It is known that potential-dependent Na^+ -channels play an important role in the maintenance and control of $\tilde{\text{Na}}^{2+}$ -homeostasis in cardiomyocytes. Na^+ ions affect the work of the $\text{Na}^+/\tilde{\text{Na}}^{2+}$ -exchanger, and its blocking affects the transport of $\tilde{\text{Na}}^{2+}$, which increases the release of $\tilde{\text{Na}}^{2+}$ from the cell during polarization, and inhibits its entry into the cell during depolarization¹⁵. These changes, in turn, affect SR Ca^{2+} content, resulting in less Ca^{2+} release in response to stimulation and reduced muscle contraction force. At the same time, in conditions where the entry of $\tilde{\text{Na}}^{2+}$ through the sarcolemma $\tilde{\text{Na}}^{2+}$ channels does not take place, Na^+ ions entering through the Na^+ channels affect

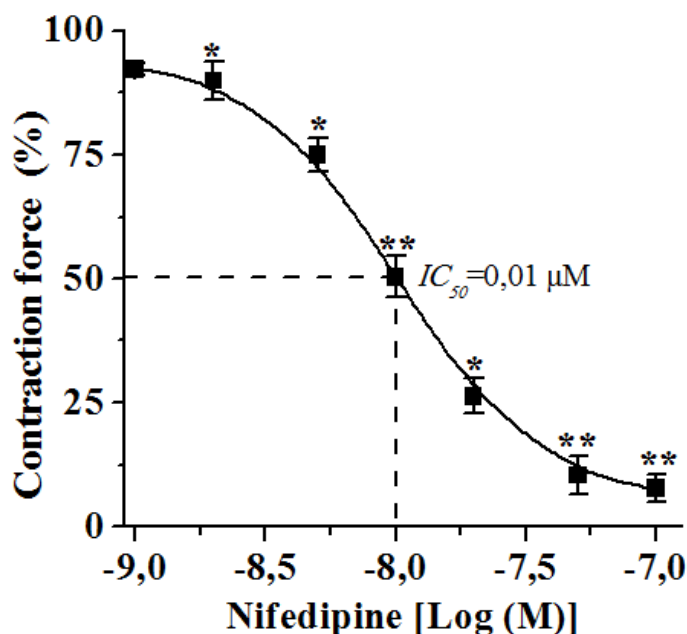


Fig. 4. Dose-dependent effect of nifedipine on papillary muscle contractile activity.

On the ordinate axis - the amplitude value of the contraction force is expressed as a percentage (%) compared to the maximum, on the abscissa axis - the concentration of nifedipine expressed in logarithm [Log(M)] is shown (*- $p < 0.05$; **- $p < 0.01$).

Stimulation frequency 1 Hz; $t = +36 \pm 0.5$ °C; $n = 4$.

the process of $\tilde{\text{Na}}^{2+}$ exit from the SR¹⁶. Under these conditions, $\tilde{\text{Na}}^{2+}$ efflux depends on $[\tilde{\text{Na}}^{2+}]_i$, suggesting the involvement of $\tilde{\text{Na}}^{2+}$ entry through the $\text{Na}^+/\tilde{\text{Na}}^{2+}$ -exchanger.

Taking into account the above, the inotropic effect of vincanine may be related to its effect on the cardiomyocyte Na^+ -channel. We tested this prediction using the Na^+ -channel blocker lidocaine. In control experiments, it was noted that lidocaine reduces the activity of muscle contraction in a dose-dependent manner

(5-30 μM). In particular, it was observed that lidocaine at a stimulation frequency of 1 Hz and a concentration of 30 μM reduces the amplitude of the papillary muscle contraction force by $83.7 \pm 2.5\%$ compared to the control (Fig. 2). In this case, the concentration of lidocaine that reduces muscle contraction by 50% (ID_{50}) is 15.4 μM .

Reduction of papillary muscle contraction under the influence of lidocaine occurs with a decrease in $[\text{Na}^+]_i$, an increase in the release of $\tilde{\text{Na}}^{2+}$ through the $\text{Na}^+/\tilde{\text{Na}}^{2+}$ -exchanger, and a decrease

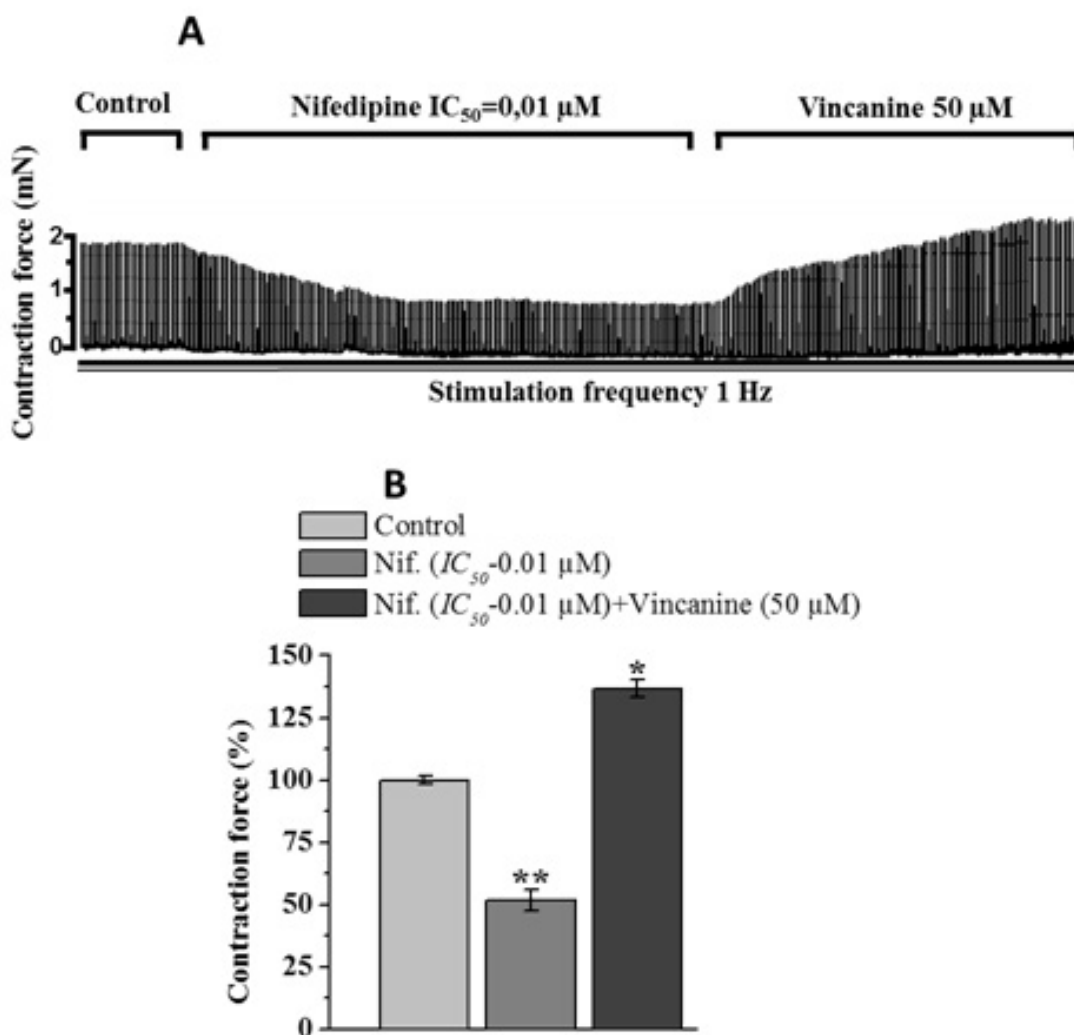


Fig. 5A, B. Effect of vincanine alkaloid on papillary muscle contraction force in the presence of nifedipine in the medium.

On the ordinate axis - the amplitude value of the contraction force is expressed as a percentage (%) compared to the maximum in all cases; **- $p < 0.01$, *- $p < 0.05$; n=5.

in $[Ca^{2+}]_i$. The addition of vincanine under these conditions did not significantly attenuate papillary muscle contraction (Fig. 3).

According to the analysis of the results of this experiment, in the presence of lidocaine, the positive inotropic effect of vincanine on papillary muscle contraction activity was preserved, and it can be concluded that this situation is not related to the effect of vincanine on the cardiomyocyte Na^+ -channel.

It is known that many agents with a positive inotropic effect lead to an increase in the of $[Ca^{2+}]_i$ in the cytosol by activating Ca^{2+}_L -channels located in the sarcolemma of cardiomyocytes¹⁷. One of the main reasons for the positive inotropic effect is the modulation of the activity of Ca^{2+}_L -channels in cardiomyocytes, which in turn is associated with the change in the amount of $[Na^+]_i$ in cardiomyocyte cells. The positive inotropic effect of vincanine alkaloid on papillary muscle contractile activity of rat heart can be caused by activation of Ca^{2+}_L -channels located in the membrane of cardiomyocytes. To clarify this assumption, the positive inotropic effect of

vincanine alkaloid was studied in the presence of Ca^{2+}_L -channel blocker-nifedipine in the incubation medium. Nifedipine exerted a negative inotropic effect at a concentration of 0.001 μ M to 0.1 μ M, and its half-maximal inhibitory effect concentration was $IC_{50}=0.01$ μ M when studying the papillary muscle contractile activity of the rat heart (Fig. 4).

In further experiments, the effect of vincanine (50 μ M) on papillary muscle contraction force was investigated under conditions of incubation of nifedipine ($IC_{50}=0.01$ μ M) in the medium. Under these conditions, the positive inotropic effect of vincanine was found to decrease by $31.6\pm 3.6\%$ compared to the control variant (Fig. 5A, B).

According to the analysis of the results of the conducted research, in the presence of nifedipine, it was found that the positive inotropic effect of vincanine and pyrazoline alkaloids on papillary muscle contraction activity was partially reduced. It can be concluded that the positive inotropic property of alkaloids is explained by their partial effect on Ca^{2+}_L -channels in cardiomyocytes.

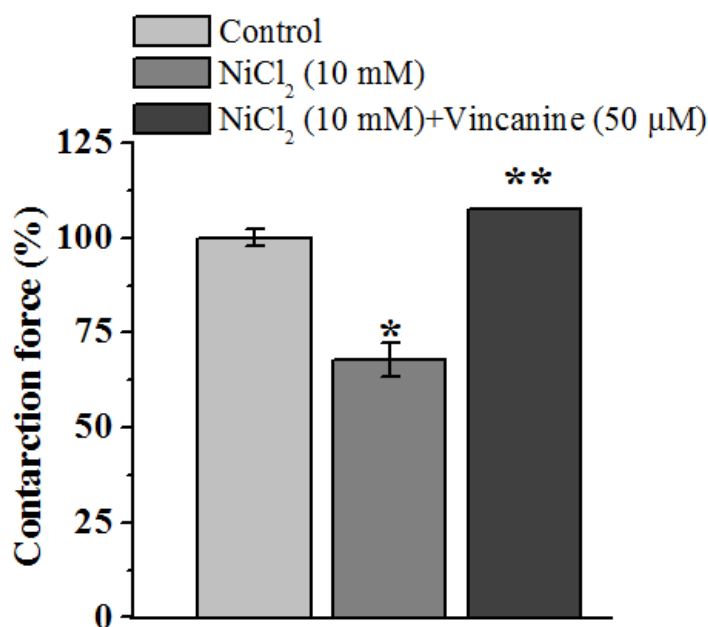


Fig. 6. Effects of vincanine in the presence of $NiCl_2$.

On the ordinate axis, the amplitude value of the contraction force is expressed as a percentage (%) compared to the maximum. The frequency of stimulation of the drug is 1 Hz. In all cases * - $p<0.05$; ** - $p<0.01$.; $n=5$.

SR $\tilde{\text{Na}}^{2+}$ -ATPase and $\text{Na}^+/\text{Ca}^{2+}$ -exchange system function play a key role in cardiomyocyte relaxation. Increased function of SR Ca^{2+} -ATPase and $\text{Na}^+/\text{Ca}^{2+}$ -exchange system leads to a decrease in $[\text{Ca}^{2+}]_i$ content in the cytosol of cardiomyocytes, which in turn increases the rate of relaxation¹⁸.

It is known that the $\text{Na}^+/\text{Ca}^{2+}$ -exchange system in cardiomyocytes in a normal physiological state is the main system that brings Na^+ ions into the cell and releases Ca^{2+} ions out of the cell¹⁹. Therefore, in the experiments, we studied the effect of these alkaloids on the papillary muscle $\text{Na}^+/\text{Ca}^{2+}$ -exchange. To evaluate the effect of vincanine and pyrazoline alkaloids on papillary muscle contraction activity and the place of $\text{Na}^+/\text{Ca}^{2+}$ -exchange located in the sarcolemma of cardiomyocytes, a concentration of 10 mM of its non-specific blocker - NiCl_2 was used. In the presence of NiCl_2 (10 mM) in the medium, the positive inotropic effect of 50 μM concentration of vincanine on papillary muscle contraction force was $107.6 \pm 4.6\%$, respectively, compared to the control (Fig. 6).

The positive inotropic effect of this alkaloid on papillary muscle contractile activity was shown to decrease by $65.3 \pm 4.1\%$ compared to the control condition. Based on this, we can explain this situation by the fact that the positive inotropic effect of vincanine modulates the $\text{Na}^+/\text{Ca}^{2+}$ exchange.

CONCLUSION

According to the obtained experimental results, it is possible to assume that the positive inotropic effect of vincanine is partly related to the Ca^{2+} ions entering the cardiomyocyte through Ca^{2+}_L -channels and mainly due to the modulation of the $\text{Na}^+/\text{Ca}^{2+}$ -exchange function in cardiomyocytes $[\tilde{\text{Na}}^{2+}]_i$ dynamics.

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Conflict of interest

The authors have declared that no conflict of interest exists.

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