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RELAXANT EFFECT OF VINCAMINE HYDROCHLORIDE AND PYROZALIN IODIDE ALKALOIDS ON AORTIC SMOOTH MUSCLE CELLS VIA CA2+ L-CHANNELS

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Abstract. *In this study, the results of the effects of vincamine hydrochloride and pyrozalin iodide alkaloids on the relaxation of aortic smooth muscle cells in the presence of $Ca^{2+}{}_{L}$-channels have been reported. The isometric contraction activity of the aortic blood vessel smooth muscle derived from rabbits was studied using mechanography. The results, under in vitro conditions, demonstrated that the contraction activity of the rabbit aortic blood vessel smooth muscle preparation induced by 50 mM KCl was dependent on the potential of the plasma membrane, associated with the activity of the $Ca^{2+}{}_{L}$-channels. It was found that vincamine and pyrozalin iodide alkaloids had an effect on the blockage of the $Ca^{2+}{}_{L}$-channels and a reduction in $[Ca^{2+}]$ concentration. The obtained results suggest that the relaxing effect of vincamine hydrochloride and pyrozalin iodide alkaloids is based on the blockage of the L-type Ca^{2+} channels, and they also affect the receptor-operated Ca^{2+} channels. In conclusion, the results indicate that in providing the relaxing effect of vincamine and pyrozalin iodide alkaloids, the blockage of potential-dependent L-type Ca^{2+} channels, along with the blockage of receptor-operated Ca^{2+} channels, plays an important role.*

Keywords: *Smooth muscle cell, aorta, ion channels, vasorelaxant.*

Introduction. The leading causes of death worldwide are heart and vascular diseases, and despite extensive efforts to prevent and manage them, they remain a significant global concern [1]. Arterial hypertension is one of the most prevalent and high-risk types among heart and vascular diseases and is considered a leading factor in both mortality and morbidity [2]. Smooth muscle cells (SMC) of blood vessels are essential components of the vascular wall and play a crucial role in regulating blood pressure. The pathogenesis of hypertension is closely related to functional and structural changes in SMC. In many cardiovascular diseases, dysfunction of ion transport systems in vascular SMC is associated with a decrease in the contractile activity of the muscle, which is crucial in regulating blood pressure [3]. Ion channels, including Ca^{2+}, K^{+}, Na^{+}, and Cl^{-} channels, are present in almost all vascular cell types, including endothelial cells, smooth muscle cells, and fibroblasts [4]. These ion channels play a significant role in regulating various physiological processes, such as cell membrane potential, signal transmission, hemodynamics, and vasomotor functions [5]. Changes in ion channels can participate in the pathologic state of the vascular system. The contractile and relaxation functions of vascular smooth muscle cells in the vascular wall are significantly influenced by the function of Ca^{2+} transport systems located on the plasma membrane and the sarcoplasmic reticulum membrane [6].

The purpose of the study. Investigation of the dose-dependent relaxant effect of vincamine hydrochloride and pyrozalin iodide alkaloids on aortic smooth muscle cells' $Ca^{2+}{}_{L}$-channels.

Research material and methods. The isometric contraction activity of the rabbit aortic smooth muscle was recorded using standard methodology (mechanography) [7]. Experimental animals were anesthetized by the dislocation method, the chest cavity was opened through a surgical incision, and the aortic blood vessel was isolated and removed. The aorta preparation was cut into ring segments of approximately 3-4 mm in length and mounted on a specialized hook in a tissue bath, and from the other side, it was connected to an isometric force measurement apparatus, Grass FT-03 (Grass Instrument, USA).

The experimental bath (5 ml) was continuously circulated with Krebs-Henseleit physiological solution, and the temperature of the physiological solution was maintained constantly by a thermostat. Additionally, the solution was aerated with a gas mixture consisting of 95 % O_2 and 5 % CO_2. The isometric contraction activity of the aortic blood vessel preparation was recorded using a Grass FT-03 mechanotransducer (Grass Instrument, USA) and a signal amplifier, Endim 621.02 (Czech Republic).

The physiological Krebs-Henseleit solution used for the continuous circulation in the experimental bath had the following composition (in mM): NaCl – 118; KCl – 4.8; $MgSO_4$ – 1.2; KH_2PO_4 – 1.2; $CaCl_2$ – 2.5; $NaHCO_3$ – 25; glucose – 11 (pH=7.4). Furthermore, the physiological solution was aerated with carbogen (95 % O_2 and 5 % CO_2), and the temperature was maintained at a constant level (t = 37±0.5 °C) using a thermostat. Initially, the rabbit aortic preparation was incubated for about 45-60 minutes under a tension of 1 g = 9.8 mN before recording the myogenic electromechanical activity.

The isometric contraction activity of the vascular smooth muscle preparation was recorded automatically using the Endim 621.02 (Czech Republic) mechanical writing apparatus. To induce the contraction of the aortic smooth muscle preparation under isometric conditions, an α_1-adrenoreceptor (α_1-AR) agonist, phenylephrine (1 μM), was used.

In the experiments, vincamine hydrochloride, pyrozalin iodide, L–NAME (Sigma-Aldrich, Germany), $NaHCO_3$, $CaCl_2$, $MgSO_4$, glucose, NaCl, KCl, NaH_2PO_4 (Russia) were used. The obtained results were statistically analyzed using Student's t-test.

Research results and their analysis. The membrane potential of smooth muscle cells (SMC) is one of the main factors in controlling their contraction and functional activity. In a resting state, the membrane potential of SMC is primarily determined by the permeability of their cytoplasmic membrane to K^+ ions. Consequently, changes in the concentration of extracellular K^+ ions lead to alterations in membrane potential, which in turn affects the functional activity of SMC. Specifically, an increase in extracellular K^+ ions in the incubation environment leads to depolarization of the SMC plasma membrane, which is associated with the development of their sustained contraction. This is why high-potassium solutions are widely used in experiments to study SMC contraction.

For instance, the contraction activity of an aortic preparation incubated in a KCl (50 mM) environment is associated with the activation of Ca^{2+} channels in the SMC plasma membrane, which is dependent on the membrane potential. In this case, an increase in the extracellular K^+ ion concentration in the environment leads to changes in the membrane potential. As a result, membrane depolarization occurs, and this, in turn, activates potential-dependent Ca^{2+} channels and

increases the intracellular Ca^{2+} ion concentration in the cytoplasm of the cells, contributing to muscle contraction.

In this context, the impact of the indole alkaloids vincamine and pyrozalin iodide on the contraction of the aortic preparation stimulated by KCl (50 mM) was investigated in these experiments. The alkaloids were found to have a potent concentration-dependent vasorelaxant effect, with concentrations ranging from 5 to 200 μM. Specifically, at a concentration of 5 μM, vincamine was observed to reduce the amplitude of aortic preparation contraction by approximately 15.7±4.2 %.

Furthermore, at a concentration of 50 μM, it was noted that this value constituted 93.6±3.6 % (Figure 1A). Similarly, pyrozalin iodide demonstrated a maximum vasorelaxant effect of 85.3±4.1 % at a concentration of 200 μM (Figure 1B).

The obtained results indicate that vincamine and pyrozalin iodide alkaloids exhibit their vasorelaxant effects through the involvement of potential-dependent Ca^{2+} channels in SMC. To further elucidate these results, in subsequent experiments, the effects of vincamine and pyrozalin iodide on $[Ca^{2+}]$out concentration were investigated. In this condition, the alkaloids were found to reduce the contraction activity of the aortic preparation by approximately 18.1±4.2 % for vincamine and 17.9±3.9 % for pyrozalin iodide (Figure 2).

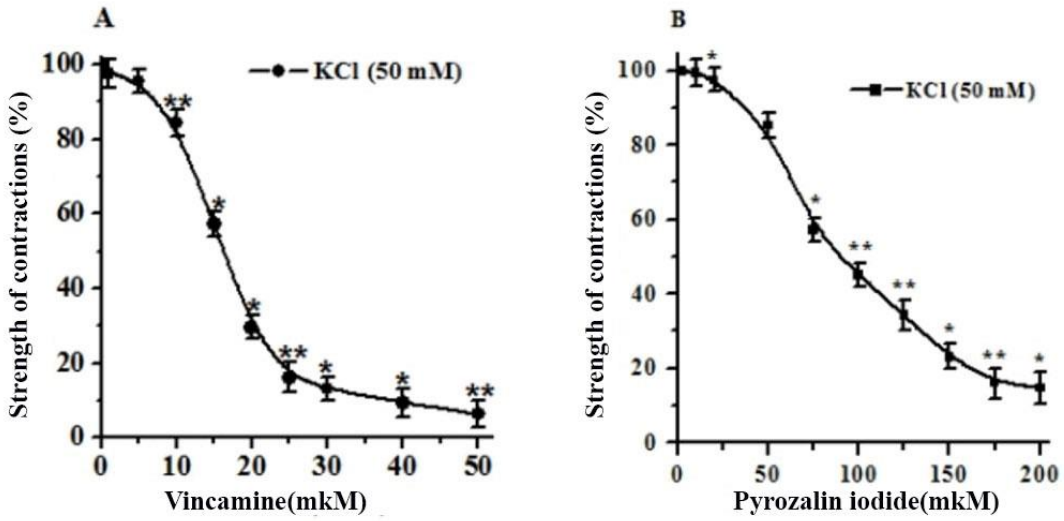

Figure 1. Concentration-dependent relaxant effects of vincamine hydrochloride (A) and pyrozalin iodide (B) alkaloids on the contraction activity induced by KCl (50 mM) in the rabbit aortic smooth muscle preparation.

*The contraction force induced by KCl (50 mM) was considered as 100% control (significant differences in all cases: *p<0.05; and **p<0.01; n=6).*

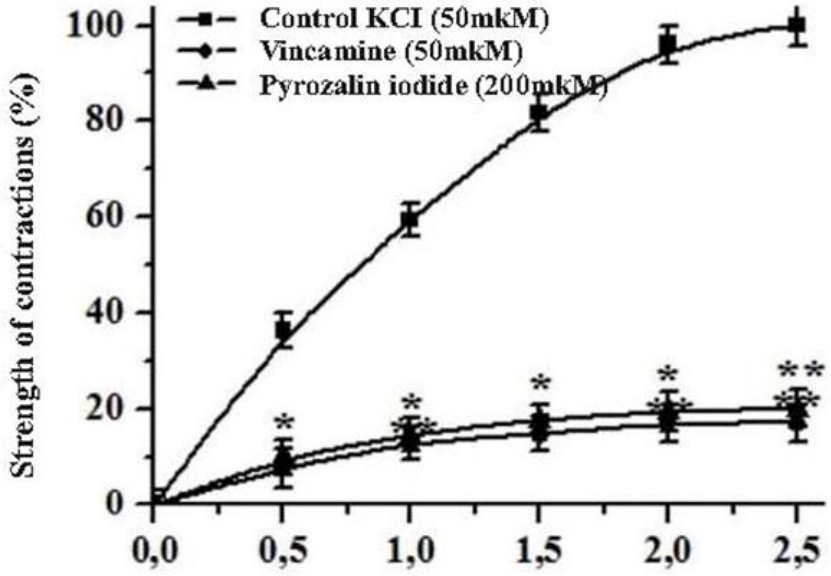

Figure 2. Relaxant effects of vincamine and pyrozalin iodide alkaloids on potential-dependent L-type Ca^{2+} channels in relation to $[Ca^{2+}]_{out}$ concentration

*The contraction force of the rabbit aortic preparation induced by cumulative addition of $CaCl_2$ to Krebs solutions in the presence of alkaloids. The contraction force induced by 50 mM KCl was considered as 100% along the ordinate axis, and the mM concentration of $CaCl_2$ was plotted along the abscissa axis (significant differences in all cases: *p<0.05, **p<0.01; n=5).*

To further clarify the above-mentioned results, additional experiments were conducted using the specific blocker of potential-dependent Ca^{2+} channels, verapamil (IC_{50} = 0.1 μM), and analyzed.

In this condition, under verapamil (0.1 μM) incubation, the contraction force of the aortic smooth muscle preparation was observed to decrease by 50±3.2 %, and under this condition, vincamine (IC_{50} = 16.3 μM) concentration further reduced the contraction force by an additional 15.5±4 %, while pyrozalin iodide (IC_{50} = 82.2 μM) led to an additional decrease of 11.9±4.2 % (Figure 3).

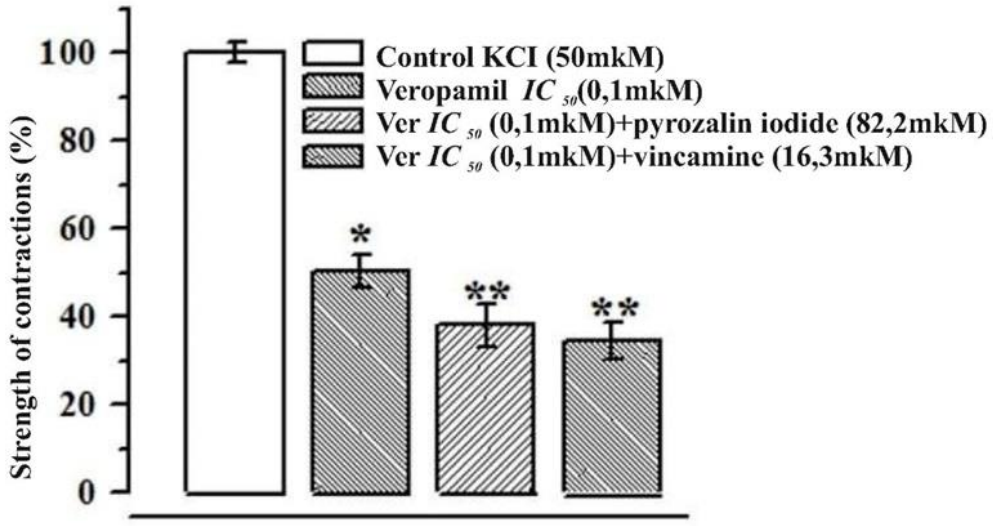

Figure 3. Relaxant effects of vincamine and pyrozalin iodide alkaloids on the contraction induced by KCl (50 mM) in the rabbit aortic smooth muscle preparation in the presence of the $Ca2^{+}_{L}$-channel specific blocker, verapamil (0.1 µM) incubation.

*The contraction force induced by KCl (50 mM) was considered as 100 % control (significant differences in all cases: *p<0.05; and **p<0.01; n=5).*

The obtained results indicate that the vasorelaxant effects of the studied indole alkaloids are associated with the blockade of Ca^{2+}_{L}-channels. Additionally, the fact that the alkaloids further reduced the effect of verapamil suggests their additional mechanism of action beyond Ca^{2+}_{L}-channel blockade. It is suggested that indole alkaloids can modulate Ca^{2+} homeostasis through the function of receptor-operated Ca^{2+} channels ($Ca^{2+}R$).

In conjunction with potential-dependent L-type Ca^{2+} channels, receptor-operated Ca^{2+} channels that are active in response to α-adrenoreceptor agonist stimulation also play a significant role in regulating Ca^{2+} homeostasis in SMC. For instance, phenylephrine (0.1-100 µM) induces contraction in blood vessel SMC through a cascade of reactions as a result of α_1-AR activation. In in vitro conditions, α_1-AR agonist phenylephrine is typically used to induce SMC contraction. After the development of contraction to a steady-state with phenylephrine, we added the alkaloids at various concentrations (ranging from 1 µM to 250 µM) and studied their relaxant effects. In the experiments, we investigated whether vincamine and pyrozalin iodide indole alkaloids play a role in relaxing the receptor-operated Ca^{2+} channels. Using the α_1-AR agonist phenylephrine (1 µM) to induce contraction, we observed that vincamine exerted a significant relaxant effect, reducing the contraction force by 18.2±4.4 % at 3 µM concentration. Vincamine had a pronounced effect on the contraction induced by 1 µM phenylephrine in the aortic preparation in the concentration range of 3-35 µM, reducing the contraction force by 95.6±3.8 % at a concentration of 30 µM, and an IC_{50} value of 12.9 µM was determined. On the other hand, pyrozalin iodide alkaloid reduced the contraction force by approximately 81.4±4.1 % at concentrations ranging from 10 to 250 µM, with a half-maximal concentration of 76.5 µM. (Figure 4).

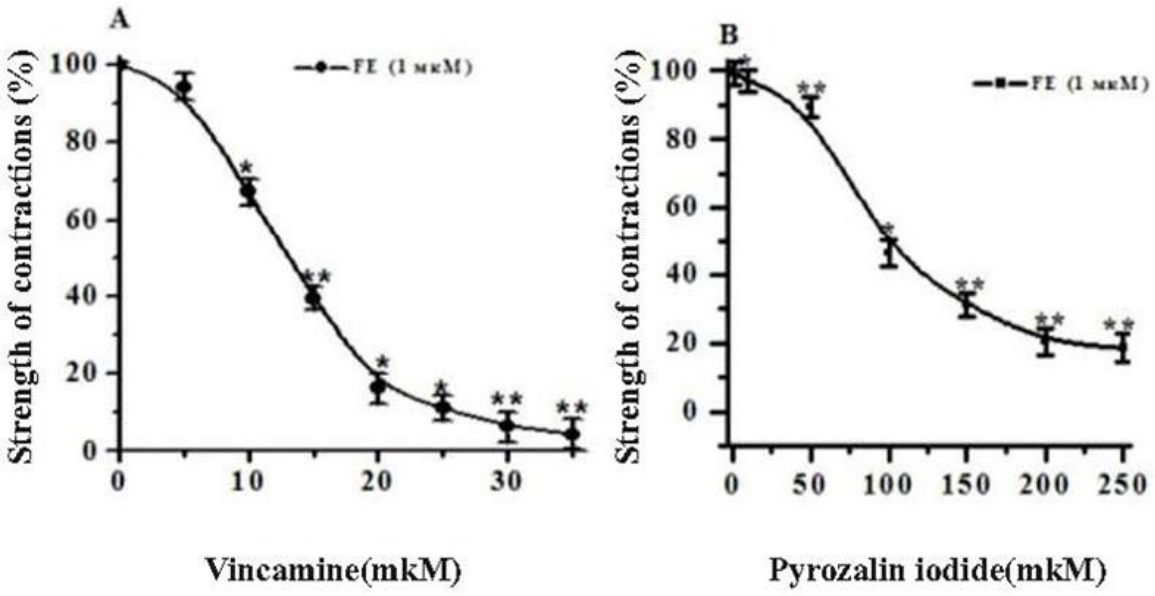

Figure 4. Effects of vincamine (A) and pyrozalin iodide (B) alkaloids on the contraction induced by phenylephrine in the rabbit aortic smooth muscle.

*The contraction force induced by 1 μM phenylephrine is considered as 100% control (significant differences in all cases: *p<0.05; and **p<0.01; n=5).*

The results obtained suggest that the observed effects of the studied alkaloids may be attributed to receptor-operated Ca^{2+} channel blockade. Such blockade of receptor-operated Ca^{2+} channels leads to reduced Ca^{2+} ion entry, a decrease in $[Ca^{2+}]_{in}$ levels, and as a consequence, relaxation of smooth muscle cells (SMC). These findings indicate that the relaxant effect of the studied alkaloids may be directly associated with the blockade of receptor-operated Ca^{2+} channels.

Figure 5 illustrates the influence of phentolamine (10 μM) on the relaxant effect of vincamine and pyrozalin iodide (5th figure). In conditions where phentolamine was not present, the relaxant effect of vincamine (35 μM) and pyrozalin iodide (250 μM) alkaloids was significantly reduced compared to the control conditions with 10 μM phentolamine, showing reductions of approximately 43.4±4.7 % and 56.7±4.3 %, respectively, under the influence of phentolamine.

These results indicate that the relaxation effect of indole alkaloids may involve both the blockade of voltage-gated L-type Ca^{2+} channels and the blockade of receptor-operated Ca^{2+} channels. Such combined effects can lead to a noticeable decrease in $[Ca^{2+}]_{in}$ levels in SMCs, potentially contributing to the significant development of the relaxant effect of the studied alkaloids.

Conclusion. The results obtained suggest that the relaxant effect of vincamine and pyrozalin iodide alkaloids on isolated rat aortic smooth muscle preparations under in vitro conditions can be explained by the activity of plasma membrane voltage-gated $Ca^{2+}{}_L$-channels that are sensitive to changes in membrane potential. It is possible to infer that their relaxant effect is associated with the blockade of L-type Ca^{2+} channels and a reduction in $[Ca^{2+}]_{in}$ concentration.

It appears that vincamine and pyrozalin iodide alkaloids exert their relaxant effect primarily through the blockade of L-type Ca^{2+} channels. However, the preservation of the relaxant effect of the studied alkaloids under the presence of verapamil implies the involvement of alternative mechanisms beyond the blockade of L-type Ca^{2+} channels in the regulation of intracellular Ca^{2+} ion levels in smooth muscle cells.

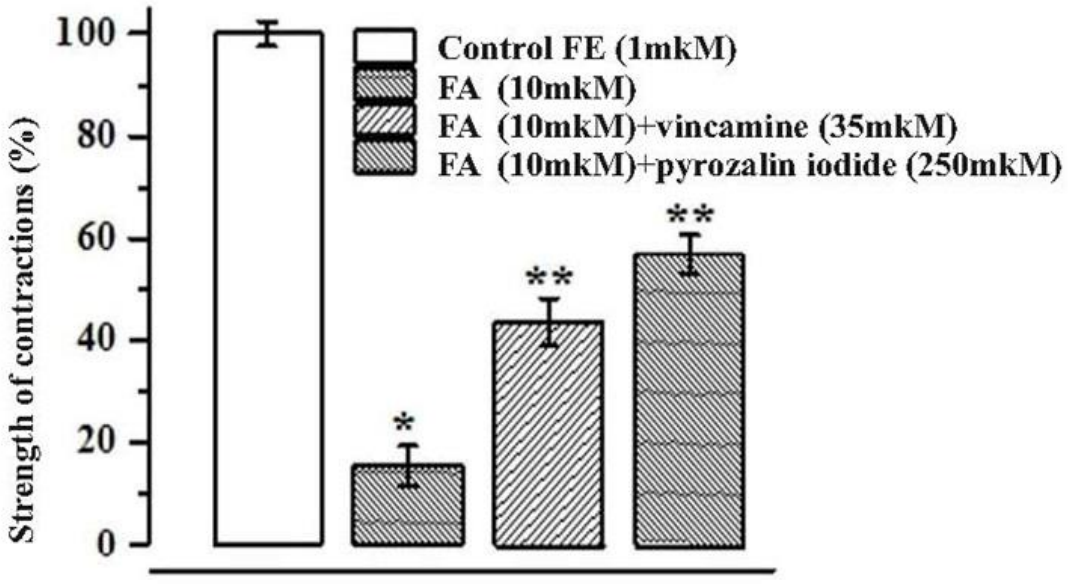

Figure 5. Influence of phentolamine (10 μM) on the relaxant effect of vincamine and pyrozalin iodide.

*The effects of alkaloids in the presence of verapamil (0.1 μM) and phentolamine (10 μM) are shown. The contraction induced by 1 μM phenylephrine is considered as 100% control (significant differences in all cases: *p<0.05; **p<0.01; n=3).*

Our experiments indicate that vincamine and pyrozalin iodide exhibit a notable relaxant effect on rat aortic preparations pre-contracted with phenylephrine, with vincamine showing a stronger relaxant effect relative to pyrozalin iodide. Consequently, our results also suggest the involvement of receptor-operated Ca^{2+} channels in the relaxant effect of these alkaloids.

These findings indicate that the relaxant effect of the studied alkaloids involves both voltage-gated L-type Ca^{2+} channel blockade and receptor-operated Ca^{2+} channel blockade. This dual mechanism may lead to a substantial decrease in $[Ca^{2+}]_{in}$ in SMCs, contributing to the significant development of the relaxant effect.

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