https://doi.org/ 10.33472/AFJBS.6.8.2024.72-78

African Journal of Biological Sciences

The effect of alkaloides of copsinine and N1-acetyl copsinine isolated from *Vince erecta* plant species on the sarcoplasmic reticulum Ca^{2+} -transport system of aortic smooth muscle

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Article History

Volume 6,Issue 8, 2024

Received:17 Feb 2024

Accepted : 28 Mar 2024

doi: 10.33472/AFJBS.6.8.2024.79-86

Abstract. The objective of this study was to evaluate the *in vitro* the sarcoplasmic reticulum Ca^{2+} -transport system of aortic smooth muscle and the underlying pharmacological mechanisms of indole alkaloides of copsinine and N1-acetyl copsinine which were isolated from the plant species *Vince erecta Regel* growing in Uzbekistan. Suspended aortic ring preparations were pre-contracted with phenylephrine (PE 1 mM), followed by cumulative addition of different concentration of alkaloids. The effect of alcaloids was partly redused by pretreatment of aortic rings preparations and even more so in endothelium-denuded aortic rings, indicating a minimal involvement of endotheliumdependent pathway in alcaloid-induced vasorelaxation. The calcium-induced vasocontractions were antagonized significantly and concentration-dependently by alcaloids in calcium free and high potassium medium. These results illustrate that Ca^{2+} antagonizing actions of alcaloids in rat isolated aorta are comparable to that of coffeinum and may be mainly responsible for its vasodilation effect.

Keywords: sarcoplasmic reticulum, inositol – 1,4,5 – triphosphate, rianodine receptor, smooth muscle, Ca^{2+} ions, contractile force.

Introduction. Cardiovascular disease is the major cause of morbidity and mortality worldwide and various cardiovascular risk factors have been identified that often exist at the same time in a patient enhancing atherosclerosis, cardiovascular morbidity, coronary artery disease and heart failure (HF) [1, 2, 3]. Agents that can act to modulate arterial wall structure and function in addition to lower blood pressure may lead to a new method in the prevention of cardiovascular morbidities [4]. The world's leading research centers have confirmed that alkaloids isolated from plants are promising sources in the production of pharmacological drugs for the prevention and treatment of diseases of the vascular system. Furthermore, alkaloids are particularly well known as anaesthetics, cardioprotective, anti-inflammatory, antioxidant and

membrane-protective agents [5, 6]. One of the main potential sources of alkaloids are isolated from the plant *Vinca erecta Regel* which have been found to have a broad spectrum of pharmacological activity. Literature data demonstrate that an infusion of the plant *Vinca erecta Regel* itself possessed hypotensive and antiarrhythmic properties [7]. Consistent continuation of scientific research in this area is of great importance from a scientific and practical point of view.

Adizov et al. isolated alcaloids copsinine and N1-acetyl copsinine from *Vinca erecta Regel* plant species and established their chemical structure [8]. However, there is few data about the biological activity of these alcaloids in the literature. The effects of these alcaloids on the vascular system have not been studied previously. Therefore, the present study was designed to examine the vasorelaxant effect of alcaloids copsinine and N1-acetyl copsinine on isolated rat thoracic aortic rings.

The aim of the study was to analyze the relaxant effect of indol alkaloids isolated from *Vinca erecta Regel* plant - copsinine, N1-acetyl copsinine and structure-activity relationship on aortic drug smooth muscle cells Ca^{2+} - transport systems.

Materials and methods. Experiments were carried out in the Laboratory of Experimental Innovative Research of the Department of Human Physiology and Life Safety of Andijan State University. The white healthy male rats (150–200 gr.) were used to carry out experiments which bred in vivarium under standard food and water conditions. All e[perimental manipulations with animals (rats) were carried out in accordance with the European Convention for the Protection of Vertebrate Animals used for experiments or for other scientific purposes.

Preparation of aortic vascular preparation was performed using a standard method [9].

The animals were anesthetized by cervical dislocation then the thorax was surgically opened and aorta was isolated. The connective tissue was removed from Isolated rat aorta preparation and put into the Krebs-Henzelight saline solution and cut into ring segments (l=2–4 mm; ø=1–2 mm). In the experimental cell (5 ml) was constantly circulated Krebs-Xenzeleite saline solution (mM): NaCl - 120.4; KCl - 5; $NaHCO_3$ - 15.5; NaH_2PO_4 - 1,2; $MgCl_2$ - 1,2; $CaCl_2$ - 2.5; $C_6H_{12}O_6$ - 11.5 (pH = 7.4). The saline solution was aerated with carbogen (O_2–95% and CO_2–5%), the temperature constant (t=+37±0.5°C) was maintained using an ultratermostat (U– 8; Bulgaria). The contractile activity of the aortic vascular preparation was recorded in isometric conditions using a power sensor FT-03 (Grass Instrument Co., USA), a signal amplifier (Grass Instrument, USA) on Endim 621.02 (Czech Republic) using a standard method (mechanography) [9, 10]. Results was statistically processed using a special software package OriginPro c. 8.5 SR1 (EULA, Northampton, MA 01060–4401, USA). Results recalculated by Laking G.F. (1990) using mathematical and statistical processing [11].

Results and discussion. Several studies have reported pharmacological effects of alcaloids on the rat cardiovascular system [12-15]. For these reasons, it appeared possible that alcaloids found in *Vinca erecta Regel* could also have cardiovascular activity. Therefore, our objective was to verify the potential vasorelaxant effect of alcaloids of medicinal plants of Uzbekistan. We studied the concentration-dependent relaxant effect of copsinine and N1-acetyl copsinine on the endothelium-intact and endotheliumdenuded aortic rings. Aorta preparation were pre-contracted with PE (1 μM) in standard K-H solution. The relaxant effect of alcaloids on the aortic rings was calculated as a percentage of the contraction in response to PE.

It is well known that in aortic smooth muscle cells, IP_3R plays a key role in the release of Ca^{2+} ions from the sarcoplasmic reticulum under the influence of mediators, hormones, and other agents. Due to an important role of IP_3R in aortic smooth muscle cells, a variety of cellular factors are involved in controlling its activity. In particular, the activity of IP_3R depends on the concentration of cellular Ca^{2+} -ions, which accelerates at concentrations up to 300 nM, and at high concentrations - inhibits it [16]. Due to these properties, IP_3R is provided with a feedback mechanism, and the release of Ca^{2+} from SR via IP_3R is controlled by the ions themselves.

However, the activity of IP_3R also is related to the level of ATP in the cell, which is activated when the amount of ATP in the cell drops to 100 μM, and at high concentrations - inhibited [17].

In this study we investigated the effect of studied alkaloids on IP_3R on aortic muscle contraction induced by phenylephrin (PE). It was obtained that the aortic muscle contracts under the influence of PE in the absence of Ca^{2+} ions condition in the Krebs solution. The contraction is due to Ca^{2+} ions emitted from the sarcoplasmic reticulum via IP_3R.

Obtained experimental data shows that the strength of aortic contraction at a concentration of 1 μM of phenylephrine in the absence of Ca^{2+} -ions in the composition of the Krebs solution was reduced by 65.3 ± 4.2% compared to the current in the Krebs solution with Ca^{2+} = 2.5 mM. In this case, the reduction occurs only due to Ca^{2+} ions emitted from IP_3R. In this medium, it was observed that the effect of copsin and N1-acetyl copsinine on the alkaloids was reduced in a dose-dependent manner. The maximum effect concentration of copsinine to reduce the tensile strength was observed at 200 μM by 18.7 ± 3.1% (Fig. 1 A), and N1-acetyl copsinine at a concentration of 100 μM by 26.8 ± 5.2% (Fig. 1 B).

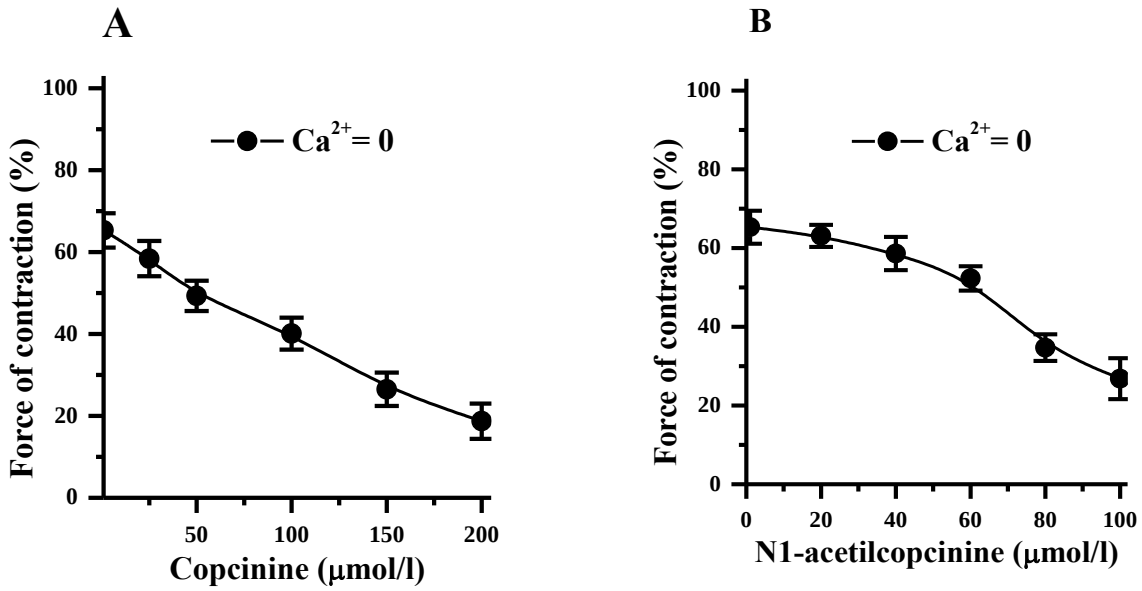

Figure 1. Dose-dependent effects of copsinine (A) and N1-acetyl copsinine (B) on aortic muscle contraction caused by PE in the Krebs solution in the Ca^{2+} = 0 state. In the presence of Ca^{2+}= 2.5 mM in the Krebs solution, the aortic contraction caused by PE (1 μM) was taken as control 100% (reliability in all cases * $p < 0.05$; n = 4).

Analysis of experimental data demonstrates that the preservation of the relaxation of aortic muscle contraction caused by phenylephrine in the absence of Ca^{2+} in the Krebs solution under the alkaloids of copsin N1-acetyl copsinine effect can be explained by the effect on the release of Ca^{2+} ions through the sarcoplasmic reticulum IP_3R.

In aortic vascular smooth muscle cells, the sarcoplasmic reticulum ryanodine receptors RyR is one of the channels that secrete Ca^{2+} ions [18]. In our experiments, we used a specific

activator of RyR -caffeine to study the effect of alkaloids on the release of Ca^{2+} ions from the sarcoplasmic reticulum. It is known that caffeine causes the release of Ca^{2+} ions into the cytosol via RyR, causing a contraction in the smooth muscle cell, and this contraction indicates the amount of all Ca^{2+} ions in the sarcoplasmic reticulum [19]. In our experiments, it was found that caffeine at the concentration of 8 mM produced a reduction of 69.8 ± 4.7% compared to the control. Dose-dependent effects of the alkaloids copsine and N1-acetyl copsine on the contraction caused by caffeine (8 mM) in the aortic muscle were observed. At a concentration of 200 μM of copsin reduced aortic muscle contraction by 21.5 ± 3.9% compared to control (Fig. 2 A), the contraction force of N1-acetyl copsin at a concentration of 100 μM decreased by 29.1 ± 3.6% compared to control (Fig. 2 B).

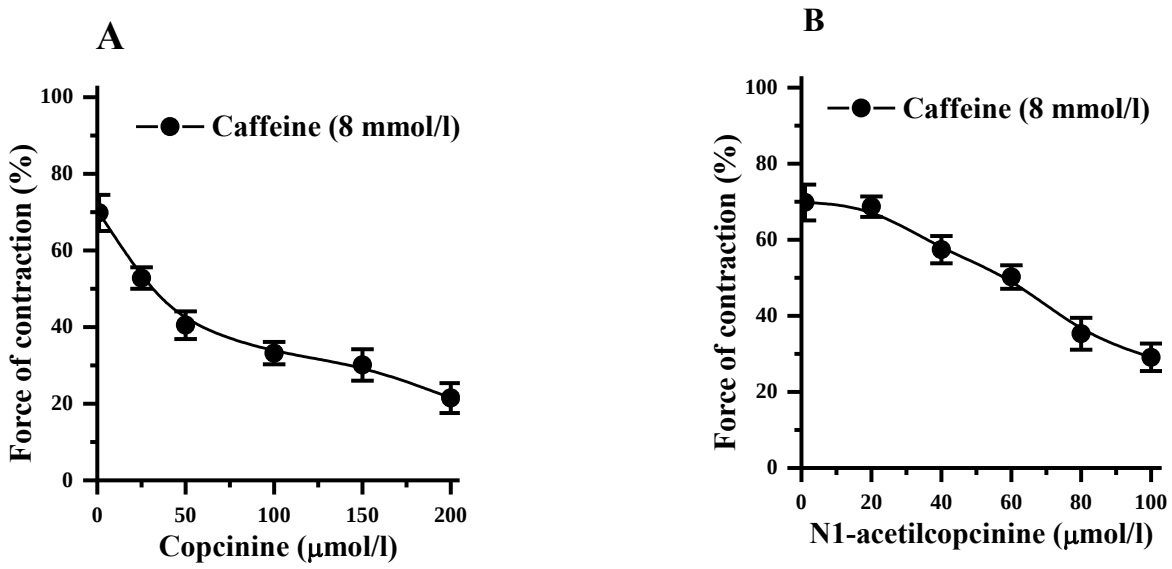

Figure 2. Dose-dependent relaxant effects of the alkaloids copsin (A) and N1-acetyl copsin (B) on caffeine-induced rat aortic contraction. The aortic contraction using PE 1 μM was taken as 100% as a control (reliability in all cases * $p < 0.05$, $n = 3$).

Analysis of the obtained results shows that the relaxant effect of the alkaloids on the aortic muscle is associated with attenuation of the release of Ca^{2+} ions through the sarcoplasmic reticulum RyR. Decreased release of Ca^{2+} ions from the sarcoplasmic reticulum under the influence of these alkaloids leads to a decrease in the amount of Ca^{2+} ions in the cytosol of smooth muscle cells, which reduces the force of contraction of the aortic muscle. The experimental results showed that the relaxant effect of the alkaloids copsin and N1-acetyl copsin on aortic muscle contraction is due to their reduction in the release of Ca^{2+} ions from the sarcoplasmic reticulum, which directly results in the relaxant effect of the studied alkaloids.

The Ca^{2+} -ATPase of sarcoplasmic reticulum in the aortic smooth muscle acts the function of pump and plays a critical role in Ca^{2+} signaling and homeostasis in all cells, collecting Ca^{2+} ions from the cytoplasm to the sarcoplasmic reticulum, thereby reducing the amount of Ca^{2+} ions in the smooth muscle cell [20]. However, in all muscles, the Ca^{2+}-ATPase of the sarcoplasmic reticulum serves as the main mechanism for the release of Ca^{2+} ions from the cytoplasm [21]. Thereby the Ca^{2+} -ATPase plays a key role in the accumulation of Ca^{2+} ions in the cells in the sarcoplasmic reticulum and in the maintenance of Ca^{2+} -homeostasis [22]. In our further experiments, we observed the effect of the alkaloids copsin and N1-acetyl copsin on the accumulation of Ca^{2+} ions in the sarcoplasmic reticulum and the effect of aortic muscle contraction caused by a blocker of the Ca^{2+} -ATPase system - cyclopiazone acid (CPA). In aortic smooth muscle cells, CPA blocks the Ca^{2+} -ATPase system of the sarcoplasmic reticulum, stopping the accumulation of Ca^{2+} ions into the sarcoplasmic reticulum, leading to an increase in the amount of Ca^{2+} ions in the cell cytosol. This causes the aortic smooth muscle to contract [23]. Based on these considerations, we used its blocker CPA to investigate the effect of the alkaloids copsin and N1-acetyl copsin on the sarcoplasmic reticulum Ca^{2+} -ATPase system. The aortic muscle contraction force under CPA (15 μM) was 64.5 ± 3.9% of the aortic muscle contraction caused by PE (1 μM). Under these conditions, a decrease in the maximum relaxing effect of the alkaloids copsin and N1-acetyl copsin was observed (Fig. 3 A and B).

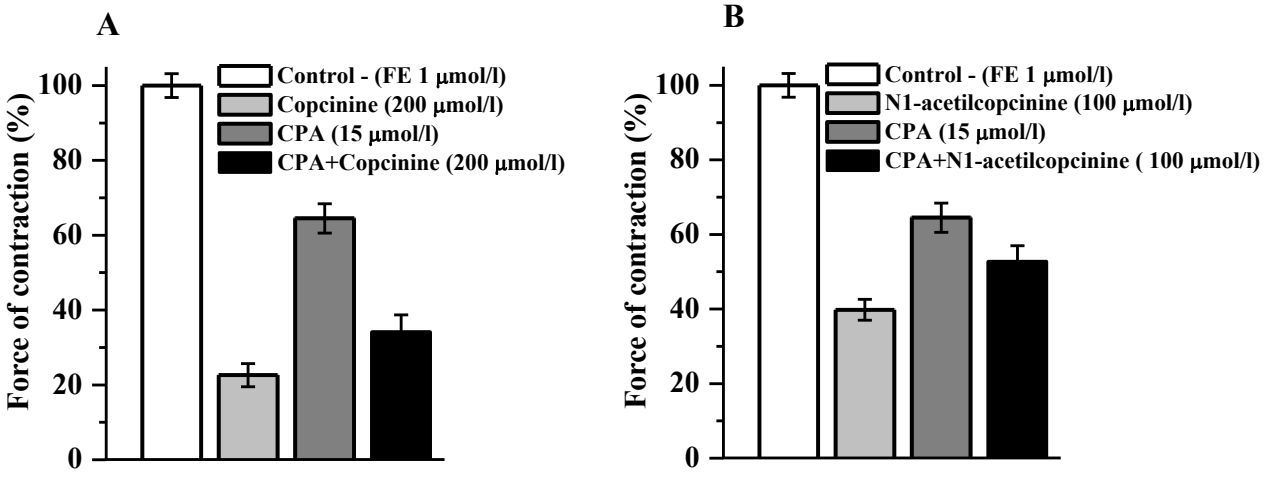

Figure 3. Relaxant effect of the alkaloids copsin (A) and N1-acetyl copsin (B) on aortic muscle contraction in the presence of cyclopiazine acid. The aortic contraction caused by PE (1 μM) was taken as 100% as a control (reliability in all cases ** $p < 0.05$; $n = 4$).

According to the analysis of the experimental results, it can be concluded that the reduction of the relaxant effect of the alkaloids on aortic smooth muscle contraction occurs as a result of inhibition of accumulation of Ca^{2+} ions in the sarcoplasmic reticulum as a result of blockade of the Ca^{2+} -ATPase system. This is confirmed by our experiments conducted above. In addition to this proposal, we studied the relaxing effect of alkaloids by pre-incubating the studied alkaloids to the aortic muscle preparation in the experiment and causing a contraction using CPA. In our experiments, a decrease in the force of contraction of the aortic muscle caused by CPA under the conditions of incubation of the alkaloids copsin (200 μM) and N1-acetyl copsin

(100 μM) was detected (Fig. 4). These results suggest that the decrease in aortic smooth muscle contraction force caused by CPA under the presence of alkaloids indicates an increase in the amount of Ca^{2+} ions in the cytosol of smooth muscle cells. In the experiment, the aortic contraction caused by TsPK was 64.5 ± 3.9%, while in the presence of alkaloids, this figure was reduced several times.

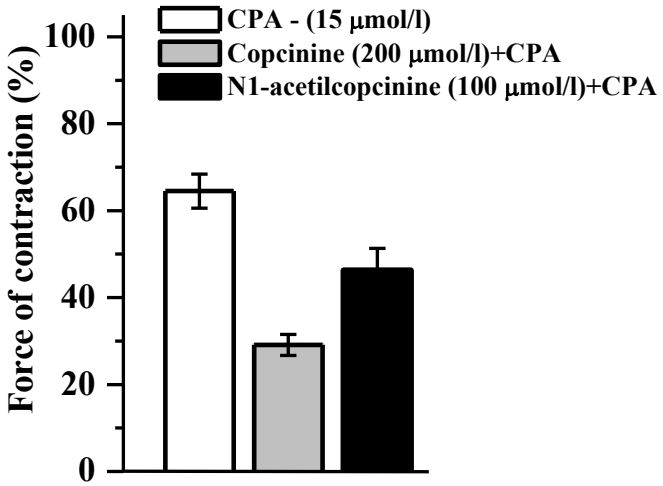

Figure 4. Aortic muscle contraction caused by cyclopiazone acid in the presence of the alkaloids copsin and N1-acetyl copsin in an incubation medium. Aortic contraction caused by CPA in the presence of alkaloids copsin (200 μM) and N1-acetyl copsin (100 μM). Muscle contraction force was taken as 100% as a control (reliability in all cases * $p < 0.05$; $n = 4$).

According to the analysis of the experimental results, we can conclude that the blockade of the sarcoplasmic reticulum Ca^{2+} -ATPase system by CPA leads to an increase in the amount of Ca^{2+} -ions in smooth muscle cells, which in turn leads to contraction of aortic smooth muscle. The relaxant effect of the studied alkaloids on aortic smooth muscle contraction is explained by a decrease in the amount of Ca^{2+} ions in the sarcoplasmic reticulum.

Conclusion. Obtained experimental data shows that the relaxant effect of the alkaloid copsin on aortic smooth muscle contractions occurs through blockade of the RyR -dependent Ca^{2+} -channel, located in the smooth muscle plasmolemma as well as the sarcoplasmic reticulum Ca^{2+} -channel. Decreased release of Ca^{2+} ions from the sarcoplasmic reticulum under the influence of these alkaloids leads to a decrease in the amount of Ca^{2+} ions in the cytosol of smooth muscle cells, which reduces the force of contraction of the aortic muscle. From the results of the above experiment, it can be concluded that the relaxant effect of the alkaloids copsin and N1-acetyl copsin on aortic muscle contraction is due to their reduction of Ca^{2+} -ions from the sarcoplasmic reticulum, which led to the relaxant effect of the studied alkaloids.

Experiments have shown that the relaxant effect of the alkaloid copsin on aortic smooth muscle contractions occurs through blockade of the receptor-dependent Ca^{2+} -channel located in the smooth muscle plasmolemma, as well as the sarcoplasmic reticulum Ca^{2+} -channel. As proof of this idea, we can explain the effect of this alkaloid on the release of Ca^{2+} ions through the sarcoplasmic reticulum IP_3R that the above-mentioned Krebs solution retains the relaxant effect on aortic muscle contraction caused by phenylephrine without Ca^{2+}.

Besides, the results of our experiments show that the relaxant effect of alkaloids of N1-acetyl copsin on aortic muscle contractions is mainly related to the effect of potential dependant Ca^{2+} L-channel located in the plasmolemma and cytosol release of Ca^{2+}-ions through the sarcoplasmic reticulum RyR. We can explain this idea with a hypercalcemic solution and the

effect of a relaxant effect that showed an alkaloid on aortic muscle contraction caused by caffeine.

The relaxant effect of the alkaloids we have studied on aortic muscle contraction may be due to modification of the accumulation of Ca^{2+} ions in the sarcoplasmic reticulum. We concluded from the analysis of the results of this experiment that the relaxant effect of the studied alkaloids on aortic smooth muscle contraction may be related to the reduction of Ca^{2+} - ions in the sarcoplasmic reticulum.

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