Western European Journal of Modern Experiments and Scientific Methods
Volume 2, Issue 10, October, 2024
https://westerneuropeanstudies.com/index.php/1
ISSN (E): 2942-1896 Open Access| Peer Reviewed

STUDY OF THE EFFECT OF SOME ALKALOIDS ON THE CONTRACTILE ACTIVITY OF AORTIC SMOOTH MUSCLE CELLS

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Key words: smooth muscle, sarcoplasmic reticulum, aorta, phenylephrine, Ca^{2+} channel, 1-O-acetylkarakoline, caffeine.

Relevance. Ca^{2+} ions play a leading role in regulation of contractile and functional activity of cardiac muscles and smooth muscles of blood vessels, which ensure normal activity of cardiovascular system as a whole [1]. In this regard, disturbances in the regulation of Ca^{2+} homeostasis are the main cause of pathogenesis of a number of diseases of the cardiovascular system, including heart failure, arrhythmias, myocardial infarction, hypertension and strokes [2]. Therefore, one of the most urgent tasks of modern biophysics, pharmacology and medicine is to study and characterize the mechanisms of pharmacological regulation of Ca^{2+}-homeostasis and Ca^{2+}-transporting systems of cardiac and smooth muscles providing its maintenance. The aim of the work was to study the effect of 1-O-acetylkarakoline, a derivative of the diterpenoid alkaloid karakolin, isolated from the plant Aconitum karakolicum, on the contractile activity of smooth muscle cells (SMC) of the rat aorta. Karakolin has a pronounced antiarrhythmic effect, which is determined by the presence of specific functionally important groups in its structure [3]. Thus, the replacement of the OH-group at the carbon atom C-1 of the lycoketonin skeleton of Karakolin with an acetyl group (Fig. 1) leads to a significant increase in antiarrhythmic activity of 1-O-acetylkarakoline [4].

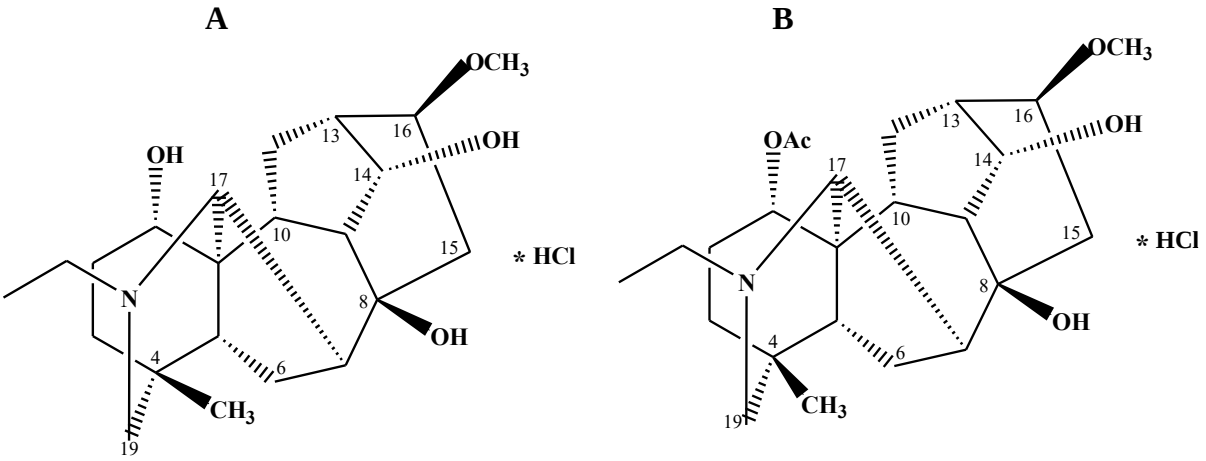

Figure 1. Chemical structure of diterpenoid alkaloids

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karakoline (A) and acetylkarakoline (B)

Research methods and materials. Isolated preparations of smooth muscle segments of aorta of white mongrel rats (200-250 g) were used in the experiments. The rats were slaughtered by cervical dislocation, the thorax was opened, the aorta was extracted and placed in Krebs physiologic solution of the following composition (mM): NaCl-120.4; KCI-5; $NaHCO_3$-15.5; NaH_2PO_4-1.2; $MgCI_2$-1.2; $CaCI_2$-2.5; $C_6H_{12}O_6$-11.5; pH 7.4. The aorta was cleared of adipose and connective tissue and cut into segments in the form of rings 3-4 mm wide. The dissected aortic segment was placed in a special experimental chamber of 5 ml volume, where it was fixed between the stem of the electromechanical transducer (FT.03, Grass, USA) and the chamber base using silver wire. The experimental chamber was perfused with oxygenated carbogen (95%O_2, 5% CO_2) Krebs solution at a constant temperature of 37˚C. Before the experiment, aortic segments were pre-stretched with a 1 g load and washed with saline for 60 minutes to achieve equilibrium. Contractions of aortic preparations were induced by applying PE (1 μM) or hyperkalemik solution (KCl, 50 mM) to the experimental chamber. Calcium-free solutions were also used in the experiments, for which Ca^{2+} ions were excluded from the Krebs solution and EGTA (1 mM) was added to bind their traces. The contractile activity of aortic preparations was recorded using a PIIT amplifier (Grass, USA) and an Endim 621.02 recorder (Germany). Statistical data processing was performed using OriginPro 7.5 program (OriginLab Corporation; USA). The contraction amplitude was expressed as % of the maximum contraction (taken as 100%) induced by phenylephrine (PE) or hyperkalemic solution and calculated as the arithmetic mean for 4-5 different experiments. Statistical significance of differences between control and experimental values was determined for a series of data using paired t-test. Values of $p < 0.05$ indicate statistically significant differences.

Results and their discussion. Previously, we found that the diterpenoid alkaloids 1-O-acetylkarakoline and karakoline effectively relax rat aortic preparations precontracted with phenylephrine and hyperkalemic solutions [5]. Therefore, the aim of the present work was to further characterize the mechanism of relaxant action of 1-O-acetylkarakoline.

To further characterize the mechanism of relaxant action of 1-O-acetylkarakoline (1-O-AK), we studied its effects on contractions of rat aortic preparations induced by the selective α-adrenoreceptor agonist phenylephrine, in the development of which Ca^{2+} ions entering the SMCs not only through potential-dependent, but also through receptor-regulated Ca^{2+}channels, and also released from the sarcoplasmic reticulum (SR) participate [6,7]. In these studies, we found. that 1-O-AK and under conditions of PE-induced contracture effectively relaxes rat aortic preparations. The relaxant effect of 1-O-acetylkarakolin was also dose-dependent, and at its concentration of 20 μM it caused relaxation of the aortic preparation by 24.5 ± 3.2%, and maximum relaxation up to 81.1 ± 4.1% was observed at its concentration of 150 μM. The magnitude (concentration воз. 50% of maximal effect) of EC_{50} of 1-O-acetylkarakolin under these conditions was 41.9 ± 4% μM. At the same time, the relaxant effect of 1-O-acetylkarakolin, under conditions of PE-induced contracture, was also observed in calcium-free solutions, the development of which is mainly provided by Ca^{2+} ions released from SR. Under these conditions, PE induced contraction of the rat aortic preparation, which was 70.4±4.1% less than the contraction induced by PE in the presence of Ca^{2+} ions. Addition of 1-O-acetylkarakolin under these conditions resulted in an additional 23.2 ± 3.8% reduction in

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contraction amplitude (Figure 2.). The results of these experiments indicate that the relaxant effect of 1-O-acetylkarakolin, in conditions of PE-induced contracture, is mainly due to its effect on the entry of Ca^{2+} ions into SMCs through receptor-controlled Ca^{2+}channels of plasmalemma and on the release from SR. It should be noted that the relaxant effect of karakolin in conditions of PE-induced contracture, as well as in conditions of KCL-induced contracture, was less pronounced in comparison with the effect of 1-O-acetylkarakolin.

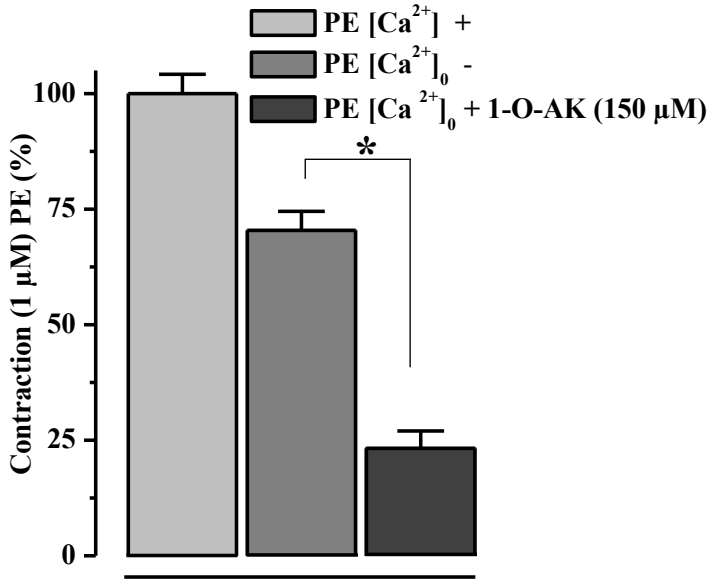

Figure 2. Effect of 1-O-acetylkarakoline on contractions of rat aortic preparations induced by phenylephrine. On the ordinate axis is the strength of aortic contraction expressed as a percentage of the control obtained by the action of 1 µM phenylephrine and taken as 100%. (P<0,05; n=5).

The results of these experiments provide convincing evidence that the relaxant effect of the alkaloid 1-O-AK is realized mainly as a result of its influence on Ca^{2+}-transporting systems of SMCs controlled by α-adrenoreceptors and related intracellular signaling cascades. It is known that stimulation of α-adrenoreceptors, inositol-1,4,5-triphosphate (IP_3R), is accompanied by activation of Ca^{2+}channels of plasmalemma and sarcoplasmic reticulum [6]. At the same time, the release of Ca^{2+} ions from SR via IP_3R contributes mainly to the increase of $[Ca^{2+}]_i$ and activation of SMC contraction. Taking this into account, we studied the effect of the investigated alkalod on the effects of caffeine, which affects the contractile activity of SMCs by specifically modifying $[Ca^{2+}]_i$ [8]. In particular, caffeine activating RyR can increase the release of Ca^{2+} ions from SR and stimulate the contraction of SMCs [9]. At the same time, caffeine can interact with IP3R and inhibiting it, suppresses the release of Ca^{2+} ions from SR and causes smooth muscle relaxation [10].

Taking into account these peculiarities of caffeine action, we studied the effect of 1-O-AK on contractions of aortic preparations induced by caffeine in the absence of Ca^{2+} ions in the incubation medium. As can be seen in Fig. 2, addition of caffeine to the solutions containing Ca^{2+} ions induced a contraction of aortic preparations, which was 67.5 ±4.1% of the contraction

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induced by phenylephrine. When Ca^{2+} ions are excluded from the incubation medium, caffeine-induced contraction of aortic preparations decreases to 33.3±3.4% of the control obtained in the presence of Ca^{2+} ions. Addition of the alkaloid 1-O-AK (150 μM) under these conditions observed an additional reduction in caffeine-induced contraction to 21.1 ± 4.4%, from control (Fig.3).

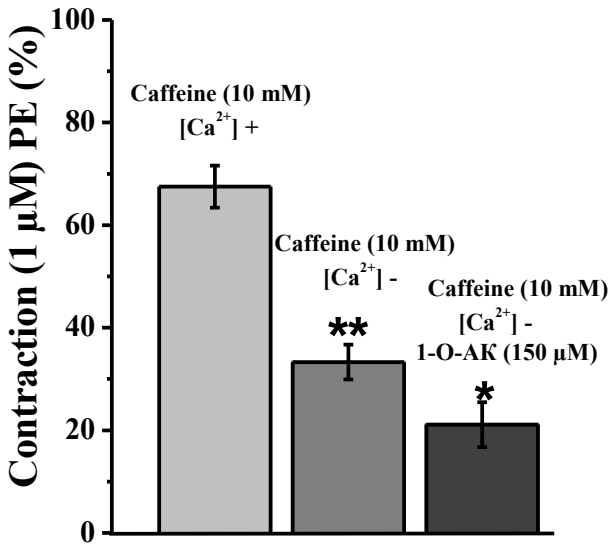

Figure 3. Effect of 1-O-acetylkarakoline on contractions of rat aortic preparations induced by caffeine. On the ordinate axis is the strength of aortic contraction expressed as a percentage of the control obtained by the action of 1 μM phenylephrine and taken as 100%, (P<0,05; n=4).

The results of these experiments indicate that 1-O-AK suppresses caffeine-induced contractions of aortic preparations. Taking into account that this effect of the studied alkaloid was detected under the conditions of absence of Ca^{2+} ions in the incubation medium, in which their entry into SMCs from the extracellular medium is excluded, we can assume that it is due to the effect of the alkaloid on the release of Ca^{2+} ions from SR via RyR or IP_3R.

Thus, analysis of the data obtained in these experiments shows that the alkaloid 1-O-AK effectively relaxes rat aortic preparations precontracted by the α-adrenoreceptor agonist phenlephrine. Taking into account that contractions induced by this antagonist are mainly caused by activation of Ca^{2+} ions release from SR via IP_3R, we can conclude that the relaxant effect of the studied alkaloid is caused by suppression of Ca^{2+} ions transport at SR level.

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