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# Vasodilator Effects of 14-Benzoilkarakoline in Isolated Rat Aorta

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**ABSTRACT**

In the current work, we looked into how the alkaloid 14-benzoilkarakoline affected the rat aorta smooth muscle's ability to contract. With the use of a force transducer, or FT-03 (Grass Instrument Co., USA), isometric tension forces were measured. 14-benzoilkarakoline has a strong relaxing effect that is mediated by a complicated network of events involving activation of the NO-guanylate cyclase system, blockage of Ca2+ influx, and their release from SR. These events result in a drop in [Ca2+]I in smooth muscle cells and their relaxation.

**Introduction.** The global public health issue of hypertension, a major risk factor for cardiovascular disease, is getting worse. Hypertension is a result of vascular smooth muscle (VSM) contraction's pathophysiological pathways. Myosin phosphorylation is caused by the balance between myosin light chain kinase and phosphatase, which triggers VSM contraction to control blood pressure. This process is regulated by calcium [$Ca^{2+}$]-dependent and - independent signaling systems [1]. In this regard, the study of the mechanisms of correction of these disorders, as well as the search for methods of pharmacological regulation of the contractile activity of the smooth muscles of blood vessels, is an urgent problem of modern physiology and medicine. At the same time, special attention is paid to natural biologically active compounds of plant origin, which have a wide range of pharmacological effects and specifically interact with various types of ion channels.

Diterpenoid alkaloids, a significant collection of naturally occurring chemicals generated by plants of the genera aconitum and delphinium, continue to provide a wide range of structural templates for drug discovery and development [2,3]. With this group, it was found that the replacement of the hydroxyl group at the C-14 carbon atom of karakolin with a benzoyl one gives its derivative 14-benzoylkarakolin a pronounced antispasmodic activity (Fig.1). 14-benzoylkarakoline (14-BK), a derivative of diterpenoid alkaloid karakoline, isolated from

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Aconitum karakolicum, has been reported to possess diverse pharmacological properties, including spasmolytic, antiarrhythmic and anesthetic activities [4, 5].

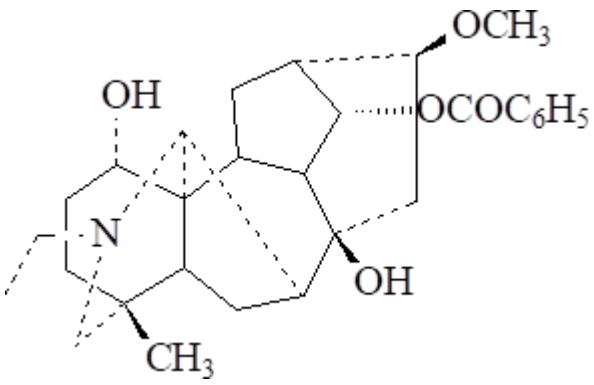



**Fig.1. Chemical structure of 14-benzoilkarakoline**

**Materials and methods Animals and aortic rings preparation**

Male albino rats weighing 200-250 g were anesthetized with sodium pentobarbital and killed by decapitation. All animal care and experimental procedures were approved by the Committee for Animal Experiments of Institute of Biophysics and Biochemistry at the National University of Uzbekistan. The thoracic aorta was quickly removed, cleaned of adherent connective tissues and cut into rings 3–4 mm in length. The aortic rings were mounted in a 5 ml organ bath contained Krebs solution of the following composition (mM): NaCl 118, KCI 4.75, NaHCO3 25, MgSO4 1.2, CaCI2 1.8, KH2PO4 1.2 and glucose 11.1, pH 7.4. Krebs solution was maintained at 37°C and bubbled with 95% O2-5% CO2 gas mixture. Aortic rings were mounted by means of two stainless steel wire hooks inserted through the lumen of the ring. Protocols of experiments on the scale and requirements of humane care of animals and the provisions of these commissions of the IBB at NUU. M. Ulugbek (Minutes No. 7 dated April 7, 2022) on the collection of laboratory animals. studies of individual segments of the aorta according to observations.

**Vascular tension monitoring**

Changes in isometric tension were recorded (with a resting tension of 1g) using a forced-displacement transducer FT03 (Grass Instrument Co. MA, USA) linked to a force transducer amplifier and saved on a computer. All studies were carried out on aortic rings with intact endothelium. Endothelial integrity was assessed by measuring the degree of relaxation caused by acetylcholine (10 M) and the contractive activity caused by phenylephrine (1 μM). Each preparation was allowed to equilibrate for at least 60 minutes before beginning the experimental procedures, and the incubation media were changed every 15 minutes during this time.

**Experimental procedures.**

Following equilibrations, the following experimental procedures were carried out: To assess 14-benzoylkarakolin's vasorelaxant activity, aortic rings were precontracted with KCl (50 mM) or phenylephrine (NA 1μM), and then 14-benzoylkarakolin was administered cumulatively to generate a concentration-response curve. To investigate the involvement of voltage-dependent $Ca^{2+}$ channels in 14-benzoylkarakolin -induced vasorelaxation, aortic rings were incubated for 30 minutes in normal Krebs solution including verapamil (0.1 μM) prior to preconstruction with KCl (50 mM) and then alkaloid were added to elicit relaxation. To determine the effect of 14-benzoylkarakolin on $Ca^{2+}$ release from intracellular storage, aortic rings were incubated in a $Ca^{2+}$free solution containing 14-benzoylkarakolin (μM) for 30 minutes before preconstruction with NA (1 μM).

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**Drugs and Solutions.**

The following drugs were used: acetylcholine, phenylephrine, verapamil, all obtained from Sigma Ltd Co. (Saint Louis, Missouri, USA). 14-benzoylkarakolin was a generous gift from Dr. M.N. Sultankhodjaev (Institute of Chemistry of Plant Substances, Tashkent, Uzbekistan).

**Statistical analysis**

Every piece of data is expressed as means S.E.M. A non-linear iterative curve-fitting approach was used to determine the concentration of 14-benzoylkarakolin that causes 50% of maximal relaxation ($IC_{50}$) and the value of maximal relaxation (Emax), which is expressed as a percentage of a prior contraction caused by the contractile agent. Statistical comparisons of mean differences were made using the Student's t-test and/or one-way ANOVA. In each test, a significance threshold of 0.05 was taken into account. Graph-Pad Prism 5.01 program (Graph Pad program Inc., USA) was used to get all values.

**Results and its discussion**

In preliminary experiments, we found that 14-benzoylkarakolin itself does not affect the basal tone of rat aortic preparations, but dose-dependently relaxes aortic preparations previously reduced by hyperpotassium solution (KCl, 50 mM), i.e. possesses a relaxing effect (Fig.2). Furthermore, in the presence of 60 μM 14-benzoylkarakolin, the amplitude of KCl-induced aortic contraction was found to be suppressed by up to 91,6±3,5% compared to controls. From these results, the $IC_{50}$ value, which is the concentration at which 14-benzoylkarakolin inhibits the decrease of 50 from the maximum value, was 16,8 μM. Considering that the expression of the KCl-evoked contractile response occurs primarily through the entry of $Ca^{2+}$ ions into the SMC via voltage-gated $Ca^{2+}$ L-type channels [6], these results suggest that the observed effects of 14-benzoylkarakolin may inhibit It may indicate that it is due to of $Ca^{2+}$ ions through these channels.

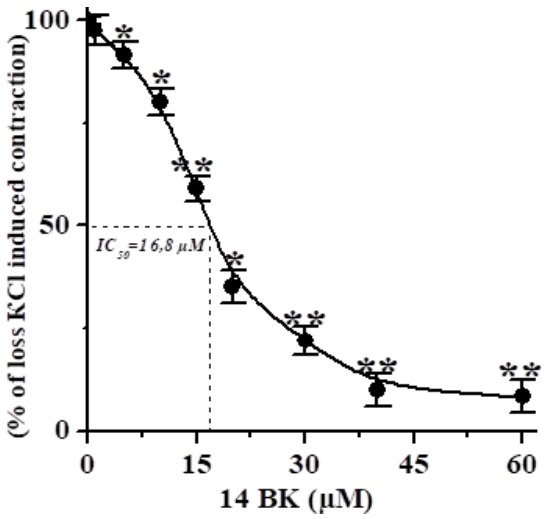



**Fig.2. Data are expressed as percentage of contraction induced by KCl (50 mM).**

Values are mean±SEM (n=5). *p<;0.05 and **p<;0.01 compared to control. To test this hypothesis, we investigated the effect of 14-benzoylkarakoline on aortic contraction induced by the addition of $Ca^{2+}$ ions to calcium-free Krebs solution containing KCl (50 mM).

These experiments found that in the presence of 14-benzoylkarakoline (60 μM), cumulative addition of $Ca^{2+}$ ions was accompanied by the generation of contractile responses, the amplitudes of which were significantly lower than controls obtained with normal Krebs solution, contains

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$Ca^{2+}$ ions. The results of these experiments indicate that the relaxing effect of 14-benzoylkarakoline is due to a reduction in intracellular levels of the ion $[Ca^{2+}]_i$ in the SMC, apparently due to inhibition of ion entry via L-type $Ca^{2+}$ channels suggests that To test this hypothesis, we examined the effects of 14-benzoylkarakoline in the presence of specific blockers of L-type verapamil $Ca^{2+}$ channels. These experiments found that addition of 14-benzoylkarakoline (16,8 μM) to the incubation medium in the presence of 0.1 μM verapamil at concentrations corresponding to $IC_{50}$ values further relaxed rat aortic preparations (Fig. 3). These results strongly suggest that voltage-gated $Ca^{2+}$ channels that are opened during depolarization are the main route by which $Ca^{2+}$ ions enter the SMC and cause the KCl-induced contractile responses of rat aortic preparations. The phase and tonic components of potassium contracture, however, were not equally susceptible to verapamil, it was discovered during these tests. At the same time, the ability of 14-benzoylkarakolin to additionally relax aortic preparations against the background of the effect of verapamil indicates that its relaxing effect is based not only on the suppression of the flow of $Ca^{2+}$ ions through voltage-dependent $Ca^{2+}$ channels, but also on its effect on other pathways. the entry of $Ca^{2+}$ ions into the SMC cytoplasm.

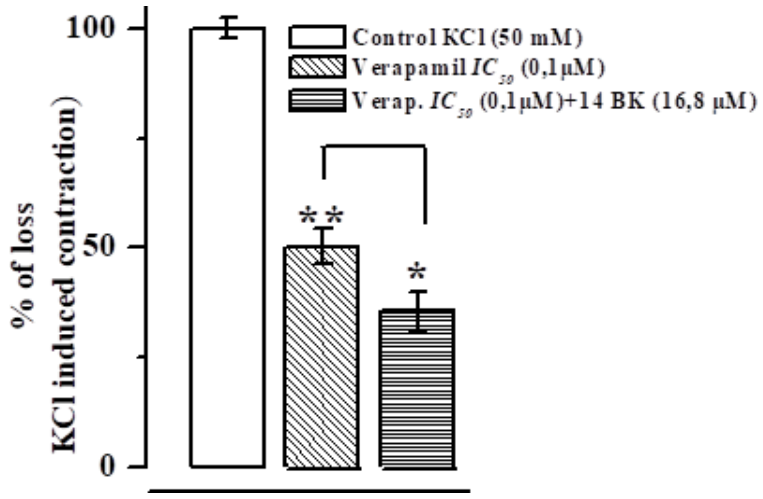



**Fig.3. Verapamil's effect on the relaxant active of 14-BK.**

The contraction force of a rat aorta preparation induced by 50 mM KCl was assumed to be 100%. (*p<0.05, **p<0.01; n=5).

In this regard, in order to further characterize the mechanism of the relaxant action of 14-benzoylkarakolin, we studied its effects on contractions of rat aorta preparations induced by the α-adrenoceptor agonist norepinephrine (NA), the development of which is provided by $Ca^{2+}$ ions entering the SMC not only through voltage-dependent, but and through receptor-gated $Ca^{2+}$ channels, as well as released from the sarcoplasmic reticulum (SR) [7]. In these studies, we found that 14-benzoylkarakolin relaxsant effectively preparations of rat aorta even under conditions of NA -induced contracture. At the same time, the relaxing effect of 14-benzoylkarakolin also had a dose-dependent character and at its concentration of 15 μM it caused relaxation of the aortic preparation by 10.2 ± 3.8%, and maximum relaxation up to 90,1 ± 4% was observed at its concentration is 100 μM (Fig.4). The $EC_{50}$ value of 14-benzoylkarakolin under these conditions was 42,2 ± 3.9% μM.

Thus, the analysis of the obtained data showed that the alkaloid 14-benzoylkarakolin has a relaxant activity and relaxes preparations of the rat aorta previously reduced with hyperkalium solution and norepinephrine.

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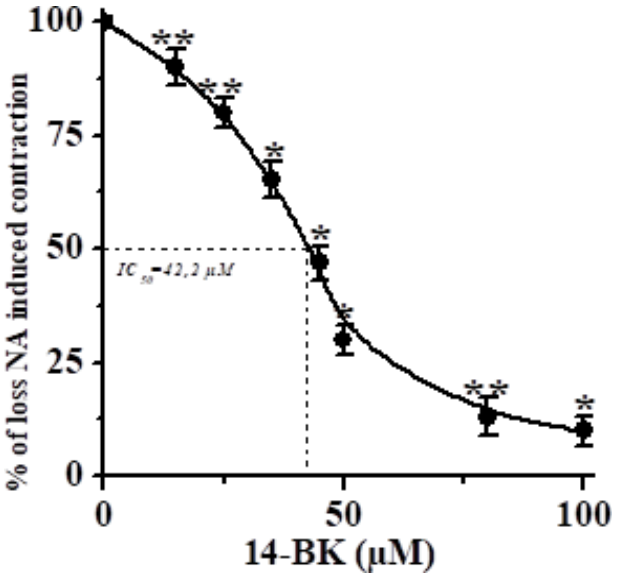



**Fig.4. Relaxant action of 14-benzoylkarakolin under conditions of NA-induced contracture of the rat aorta.**

Dependence of the relaxant action of 14-benzoylkarakolin on its concentration. The force of contraction of the rat aorta preparation induced by 1 μM NA was taken as 100%. ($P<0.05$; n=5).

These findings suggest that a number of mechanisms, including the blockade of voltage-dependent $Ca^{2+}$ channels (during KCI-induced contracture) and the inhibition of $Ca^{2+}$ ion transport through receptor-gated $Ca^{2+}$ channels and their release from SR (during NA-induced contracture), are involved in the implementation of 14-BK relaxant action.

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