CHARACTERIZATION OF THE MECHANISM OF ACTION OF NAJA OXIANA EICHWALD POISON FRACTIONS ON CARDIAC AND SMOOTH MUSCLE CONTRACTILE ACTIVITY.

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Abstract: A dose-dependent effect of naja oxiana eichwald venom S-6 and S-7 fractions on cardiac muscle contractile activity was investigated, and it was found that they have a negative inotropic effect on muscle contraction force. S-6 fraction at a concentration of 2 μg/ml was observed to reduce cardiac muscle contraction force to 8.3±3.4% and S-7 fraction to 26.1±3.4% at 5 μg/ml. The suppressive effect of these fractions was maintained in the presence of nifedipine, but to a slightly lesser extent due to the blockade of Ca^{2+}-channels accompanied by a decrease in the level of $[Ca^{2+}]_i$ ions in cardiomyocytes. Blockade of Na^+ channels with lidocaine also led to a noticeable suppression of the negative inotropic effect for fractions S-6 to 36.3±4.2% and S-7 to 41.6±4.4% of the control, respectively. Also, it was found that the effects of S-6 and S-7 fractions on rat aorta smooth muscle contraction strength were investigated. Considering that KCl-induced contractions of the rat aorta are mainly ensured by the activation of voltage-dependent Ca^{2+}-channels, the observed effect of fractions S6 and S7 may be associated with inhibition of Ca^{2+} ion transport through these channels. The influence of fractions S6 and S7 of cobra venom on the functioning of voltage- and receptor-dependent Ca^{2+}-channels in the rat aorta and cardiac muscle was studied.

Keywords: cardiac muscle, smooth muscle, Ca^{2+}-channels, naja oxiana eichwald, fraction**.**

INTRODUCTION

Pathologies of the cardiovascular system occupy a leading place in the overall structure of morbidity and mortality throughout the world [1]. The development of these cardiovascular diseases is based on a complex of common pathogenetic processes that disrupt the transport function of the heart artery, energy metabolism in cardiomyocytes, and disruption of the function of Ca^{2+}-transport systems, and in general, lead to a violation of the functional activity of the myocardium. [2]. Although significant progress has been made in the treatment and prevention of hypertension, ischemic heart disease, heart failure, and cardiac arrhythmias in recent decades, the issue of effective and complete treatment of these diseases has not yet been resolved. [3]. This situation is largely because most of the drugs used in the treatment of these diseases are underdeveloped and have significant shortcomings, which in turn significantly limits their therapeutic effectiveness and wide-scale use. All this implies the need to develop new, adequate approaches to the treatment and prevention of these diseases, as well as effective means for pharmacological protection of the myocardium from damage based on the latest achievements in the

field of studying the molecular mechanisms underlying the pathogenesis of these diseases. Also, animal poisons consist of a complex mixture of components that affect several biological systems. Snake venoms, the composition of which has been studied at the level of peptides, are widely used in medicine and pharmacology. Currently, in many countries of the world, scientific research is being conducted on the creation of analgesic and antimicrobial drugs based on snake venoms [4, 5, 6]. Snake venoms, composed of a mixture of complex biologically active substances, differ among themselves according to the mechanism of their effect on the human body: the venom of the spectacled snake (cobra or naja oxiana eichwald) affects the function of the human nervous system, and the venoms of the gyurza and black snakes (gyurza and viper) mainly, it affects the walls of blood vessels and the cardiovascular system [7, 8,]. However, the mechanism of action of cobra venom fractions on heart and smooth muscle cells has not been studied. Taking this into account, the effect of cobra snake venom fractions on rat heart papillary muscle and aortic vascular smooth muscle cells was studied.

MATERIALS AND METHODS

Rat cardiac papillary muscle under isometric conditions is a method of studying contractile activity. Researches were carried out in the laboratory of cell biophysics of the Institute of Biophysics and Biochemistry under National University of Uzbekistan. Purebred, white rats (100–250 g) were used in the experiments, and the International Helsinki Declaration and the International Council of Medical Scientific Societies (CIOMS) (1985) rules for working with experimental animals were followed. The experimental animals were anesthetized with diethyl ether, euthanized by cervical dislocation, and the chest cavity was surgically opened, the heart was removed, and papillary muscle preparation (ø=~0.4–1.3 mm; l=~ 2.5–3.8 mm) was prepared and recorded the contraction activity of the papillary muscle preparation using a mechanographic device (Mayflower Tissue Bath System, Hugo Sachs Electronic, Germany) and a hardware-software complex (LabScibe 2, World Precision Instruments, USA). The papillary muscle isolated from the right ventricle of the rat heart is placed in a 5 ml chamber connected to a thermostat (36±1°C), continuously perfused with Krebs physiological solution containing oxygenated carbogen (O_2–95% and CO_2–5%) with the following composition: NaCl – 150; KCl – 4; $CaCl_2$ – 1.8; $MgCl_2$ – 1; $NaHCO_3$ – 14; NaH_2PO_4 – 1.8; $C_6H_{12}O_6$ – 11.5 (pH=7.4). Before the start of the experiment, it is stretched to 30% of its original length and adapted for 60 minutes to obtain maximum contraction. Stimulation of the muscle was carried out with the help of 2 silver electrodes located along the entire length of the chamber, with a frequency of 1 Hz with an amplitude of 2 times the step level, a right-angled electrical impulse with a duration of 5 ms.

Method of recording the contraction of the rat aortic vascular preparation. Experimental animals were euthanized by cervical dislocation, and the chest was opened, the aorta was surgically isolated, and Krebs-Henseleit physiological solution ((мМ): NaCl-120.4; KCl-5; $NaHCO_3$-15.5; NaH_2PO_4-1.2; $MgCl_2$-1.2; $CaCl_2$-2.5; $C_6H_{12}O_6$ -1.5; pH-7.4.) was placed in a special chamber (5 ml). The physiological solution was aerated with carbogen (O_2–95% and CO_2–5%), and temperature constant (+37±0.5°C) was ensured using an ultra thermostat (U–8; Russia). The

contractile activity of the aortic vascular preparation was connected to the FT-03 sensor (Grass Instrument Co., USA) in isometric conditions using hooks made of platinum wire and recorded by a special program on a computer through a signal amplifier device (Grass Instrument, USA).

RESULTS

Study of the effects of fractions on the contractile activity of the papillary muscle of the rat heart. As can be seen in Fig. 1 A and B, fractions S-6 and S-7 dose-dependently suppress the force of contraction of the papillary muscle. The effect of fraction S-6 on papillary muscle contraction was more pronounced and it maximally suppressed the force of muscle contraction to 8.3±3.4% of the control level at a concentration of 2 μg/ml (Fig. 1 A). At the same time, the EC_{50} value for fraction S-6 was 1.1 μg. Whereas, in the presence of the S-7 fraction, the maximum suppression of muscle contraction force to 26.1±3.4% of the control level was observed at its concentration of 5 μg/ml (Fig. 1.B). The EC_{50} value (the concentration of the fraction causing 50% of the maximum effect) was 3.12 μg/ml.

The results of these experiments show that the studied fractions significantly suppress the force of contraction of the heart muscle, which indicates that they have pronounced negative inotropic activity.

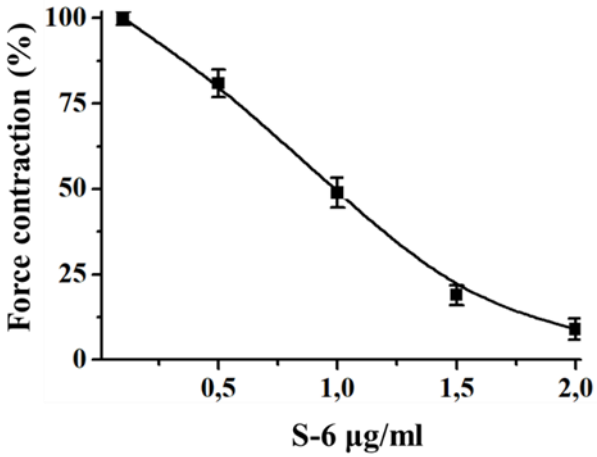

Fig.1 A. Dependence of the effect of fraction S-6 on papillary muscle contraction on its concentration. Along the ordinate axis is the force of muscle contraction expressed as a percentage of the control taken as 100%. Stimulation 0.5 Hz (t=+36±0.5ºC). p<0.01 (n=4).

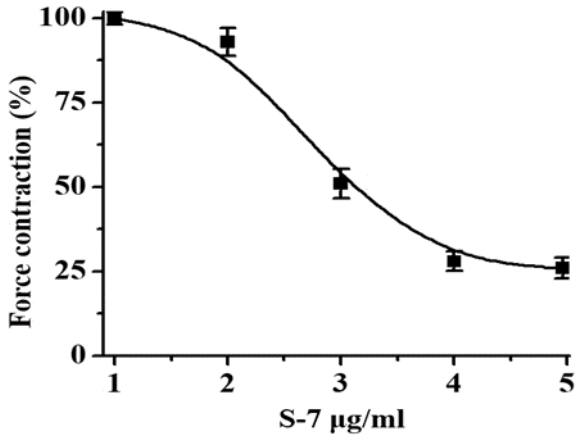

Fig.1 B. Dependence of the effect of the S-7 fraction on the contraction of the papillary muscle on its concentration. Along the ordinate axis is the force of muscle contraction expressed as a percentage of the control taken as 100%. Stimulation 0.5 Hz (t=+36±0.5ºC). p<0.01 (n=4).

The negative inotropic effect of most inotropes is due to a decrease in the intracellular level of $[Ca^{2+}]_i$ ions in cardiomyocytes, which occurs as a result of suppression of their entry through voltage-dependent Ca^{2+} channels of the sarcolemma

In this regard, to assess the effect of the studied fractions on the transport of Ca^{2+} ions through Ca^{2+}-channels, their effects were studied in the presence of the blocker of these channels, nifedipine. It was found that in the presence of nifedipine (0.01 μM, a concentration corresponding to its EC_{50}), the studied fractions retained the ability to suppress the force of muscle contraction, but not to the same extent as in the absence of nifedipine (Fig. 2). Under these conditions, fractions S-7 and S-6 reduced the force of muscle contraction to 28.7±3.9% and 19.3±4.2% of the control, respectively.

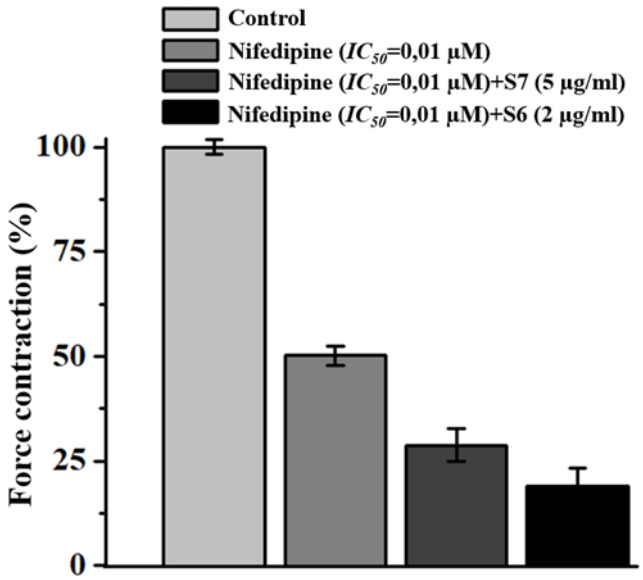

Fig.2. Effect of nifedipine on the negative inotropic effect of fractions S-6 and S-7. Along the ordinate axis is the force of muscle contraction expressed as a percentage of the control taken as 100%. Stimulation 0.5 Hz (t=+36±0.5ºC). p<0.05 (n=4).

The results of these experiments may indicate that the negative inotropic effect of the fractions is achieved due to the blockade of Ca^{2+}-channels accompanied by a decrease in the level of $[Ca^{2+}]_i$ ions in cardiomyocytes. However, the partial preservation of the negative inotropic effect of fractions in the presence of nifedipine, as well as a decrease in its maximum, indicates the participation of other ion channels in its provision.

In this regard, to test the role of other ion channels in ensuring the negative inotropic effect of the studied fractions, their effects were studied in the presence of lidocaine, a blocker of voltage-dependent Na^+-channels. In these experiments, it was found that the blockade of Na^+-channels with lidocaine also leads to a marked suppression of the negative inotropic effect of the studied fractions. However, in the

presence of lidocaine, fractions S-7 and S-6 reduced the force of muscle contraction to only 41.6±4.4% and 36.3±4.2% of the control, respectively (Fig. 3).

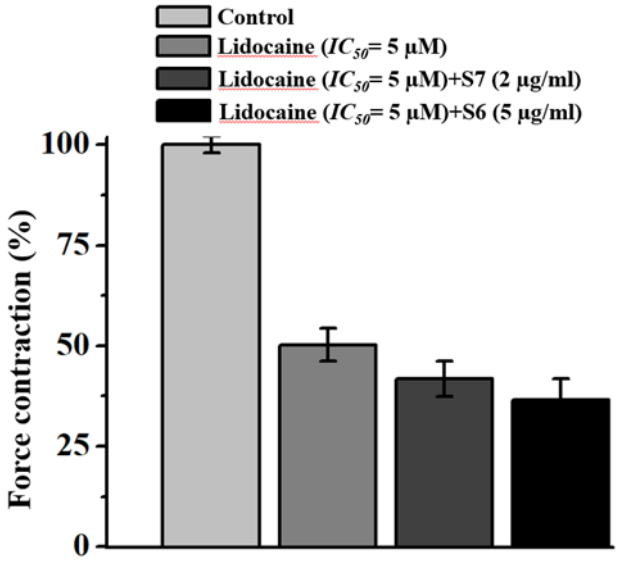

Fig.3. Effect of lidocaine on the negative inotropic effect of fractions S-6 and S-7. Along the ordinate axis is the force of muscle contraction expressed as a percentage of the control taken as 100%. Stimulation 0.5 Hz (t=+36±0.5ºC). $p<0.05$ (n=4).

These results indicate that the blockade of voltage-dependent Ca^{2+} and Na^{+}-channels of cardiomyocytes plays a leading role in providing a negative inotropic effect (reducing the force of contraction) of the studied fractions, which, is a possible mechanism for the cardiotoxic effect of some cytotoxins [9, 10], obtained from cobra venoms.

Study of the influence of fractions on the contractile activity of the aorta of the rat heart.

It is known that Ca^{2+}-channels are activated exclusively through the receptor-mediated pathway, and not as a result of depolarization of the plasma membrane characteristic of snake venom cytotoxins. Therefore, to elucidate the possible mechanism of action of fractions S7-S6 of cobra venom, their effect on the functioning of voltage-dependent Ca^{2+}-channels in the aorta and cardiac muscle of the rat was studied (Fig. 4). Using this model, the inhibitory effect of the materials under study was revealed. So, IC_{50} for fractions S6 and S7 in the case of voltage-dependent Ca^{2+}-channels are in the range of 30-40 μg/ml. Considering that KCl-induced contractions of the rat aorta are mainly ensured by the activation of voltage-dependent Ca^{2+}-channels , the observed effect of fractions S6-S7 may be associated with the inhibition of Ca^{2+} ion transport through these channels.

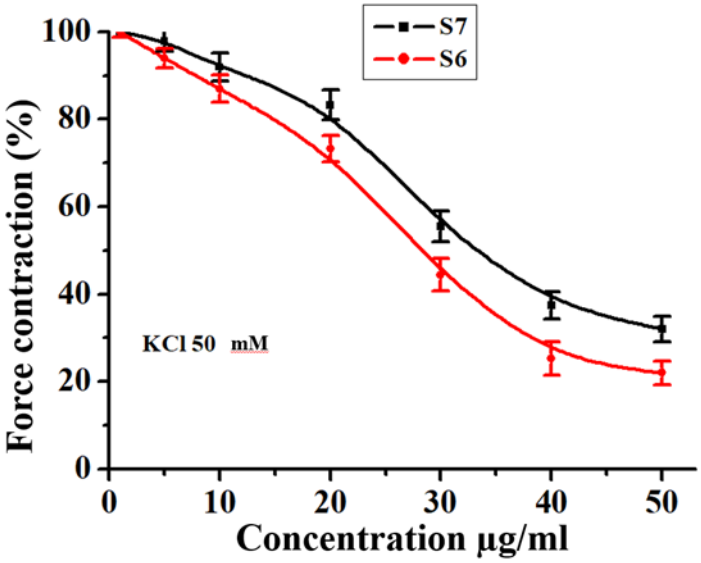

Fig.4. Effect of fractions S6-S7 on KCl-induced contraction of the rat aorta (voltage-dependent Ca2+ channel). On the ordinate, the aortic contraction force evoked by KCl 50 mM was taken as 100%. Concentrations of fractions S6-S7 are shown on the abscissa (in all cases, confidence index $p<0.05$; $n=4$).

When studying the effect of these fractions on receptor-dependent channels induced by phenylephrine, inhibition was observed at lower concentrations: 20-25 µg/ml, respectively (Fig. 5).

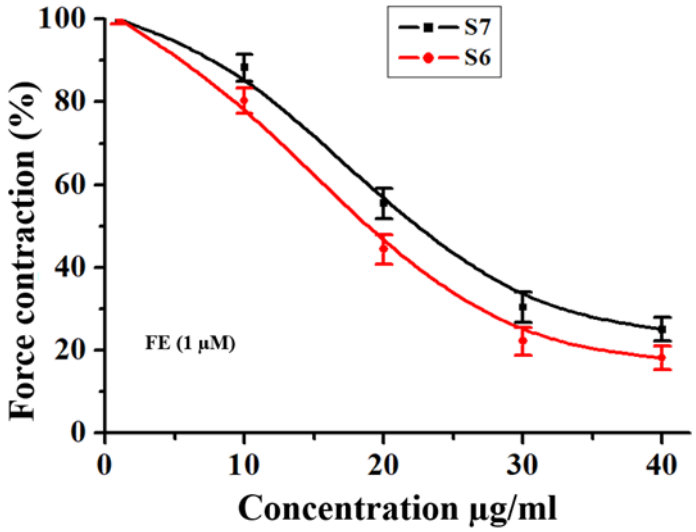

Fig.5. Effect of fractions S6-S7 on phenylephrine-induced contraction of the rat aorta (receptor-gated Ca^{2+} channel). On the ordinate, aortic contraction force evoked by phenylephrine 1 µM was taken as 100%. Concentrations of fractions S6-S7 are shown on the abscissa (in all cases, confidence index $p<0.05$; $n=4$).

The effect of fractions S6-S7 on phenylephrine-induced aortic contractions, which are mediated by Ca^{2+} ions entering through receptor-gated Ca^{2+}-channels and released from the sarcoplasmic reticulum, indicates a possible effect on these Ca^{2+} transport systems.

CONCLUSION

The effect of fractions S-6 and S-7 of cobra venom on the functioning of voltage- and receptor-dependent Ca^{2+}-channels in the rat aorta and cardiac muscle was studied. In both models, the inhibitory effect of the studied materials was revealed. So, EC_{50} for French. S-6 and S-7 in the case of voltage-gated Ca^{2+}-channels are in the range of 30-40 μg/ml, while receptor-gated channels induced by phenylephrine were inhibited at lower concentrations: 20-25 μg/ml, respectively. Fractions S-6 and S7 also had a suppressive effect on the force of contraction of the heart muscle (EC_{50} fraction S-6 is 1.1 μg/ml; EC_{50} fraction S-7 is 3.12 μg/ml). The suppressive effect of these fractions was maintained in the presence of nifedipine but to a slightly lesser extent due to the blockade of Ca^{2+}-channels accompanied by a decrease in the level of $[Ca^{2+}]_i$ ions in cardiomyocytes. Blockade of Na^{+}-channels with lidocaine also led to a noticeable suppression of the negative inotropic effect for fractions S-6 to 36.3±4.2% and S-7 to 41.6±4.4% of the control, respectively. It has been suggested that the observed effects of the analgesic action of the components of cobra venom may be a consequence of the inhibition of voltage-dependent Ca^{2+} and Na^{+}-channels, indicating possible mechanisms of their action through modulation of the functioning parameters of these channels.

Conflict of interest. The author declares that he has no conflict of interest.

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