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## BIOLOGICALLY ACTIVE PHENOL AND INDOLE DERIVATIVES OF TERRESTRIAL ARTHROPODS: ELECTROPHYSIOLOGICAL AND CHEMICAL CHARACTERISTICS

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Toxins-antagonists of glutamatergic synapses were found in venoms of different terrestrial *Arthropodae*, both *Insecta* and *Aranei*. Chemical structures of some of them were described, and a majority of such substances are derivatives of phenol and indole. They are used successfully for electrophysiological investigations of membrane structures, and present review is devoted to some results of such studies that may be useful for laboratory investigations. Here, we described chemical structure of some toxins, as well as the results of electrophysiological registration of activities of different venoms and toxins of *Arthropodae*. Hypotheses that explain physiological effects of the substances – antagonists of glutamate receptors depending on the peculiarities of their chemical structures are suggested. The role of different sites of toxins' molecules in blocking of glutamate receptors is discussed. The information about venoms' components of *Insecta* family *Sphecidae* (*Philanthus triangulum*), and of some *Araneidae* spider species of *Argiope*, *Araneus*, *Nephila* genera is also presented.

**Keywords:** Arthropods' venoms, toxins, receptor antagonists, transmembrane electric current

Arthropods' toxins are highly specific natural substances; some of them are phenol and/or indole derivatives. Toxins-antagonists of the glutamatergic synapses are used for the electrophysiological investigations of membrane structures. Such glutamate receptors' antagonists of natural origin were also found in venoms of different terrestrial Arthropods, both *Insecta* and *Aranei*. Chemical structures of some of them were

deciphered as phenol or indole derivatives. Here, we described chemical structure of the most important arthropods' toxins, as well as some results of electrophysiological studies of the biological activities of their venoms and toxins. The information about venomous components of predatory *Insecta* from family *Sphecidae* (*Philanthus triangulum*), and some spider species from genera *Argiope*, *Araneus*, *Nephila* (fam. *Araneidae*) are also presented. Taking into consideration these data, we proposed the hypotheses that explain physiological effects of studied substances, namely the antagonists of glutamate receptors, depending on the peculiarities of their chemical structures. Finally, the role of different sites of toxins' molecules in blocking of glutamate receptors is discussed.

Investigations of potential glutamate receptors' antagonists obtained from venoms of different terrestrial *Arthropodae* primarily of *Argiope* and *Araneus* genera have started actively in 1980th, and they stay promising for studying of the glutamate receptors [16, 20, 29, 59]. It is known that not only spiders (*Araneidae*) are producers of toxins – antagonists of glutamate receptors, but some other *Arthropodae* do that as well. A spectrum of such organisms and, respectively, their toxins are extremely wide. Brief information on the properties of venoms and toxins of the arthropods was presented in [2, 36, 39, 48–50]. Here, we provide more information on the results of experimental studies of glutamate receptors antagonists from different arthropods' venoms, as well as the results of studies of chemical structure of some toxins obtained from these venoms. The results of studying different natural toxins for the neurophysiologic purposes are well described in the monographs by P.G. Kostyuk, O.O. Krishtal, I.S. Magura, and I.V. Skok [58, 63, 81]. These investigations were continued by the representatives of scientific schools headed by the above mentioned scientists in collaboration with their foreign partners [2, 36,]. Nowadays, the results of study of specific toxins from *Arthropodae* (including *Araneidae* toxins), as well as other similar phenol and indole derivatives were applied in agriculture [16, 20, 29, 59] and in the methods of ecological monitoring of environment [38–57]. Some of these results were supported by the patents [46–57]. Our review is devoted to *Arthropodae* toxins' study and application [1–19, 21–37, 60–97]. Study of chemical structure of arthropods' toxins allowed to define their possible role, as well as the role of their fragments in blockage of different channel-receptors complexes (CRC) [5, 8, 12, 22, 23, 28, 35, 37, 40].

**Antagonists of glutamatergic synapses in venoms of different terrestrial Arthropods and experimental study of their biological activity.** At the end of 20th century, the investigators of different countries started isolation and study of the properties of some antagonists of glutamate receptors [2, 36, 24–92]. The producers of such antagonists were found in spiders of seven species living on the territory of former Soviet Union: *Agalenatea redii*, *Mangora acalypha*, *Neoskona adianta*, *N. cruciferoides*, *Nuctenea folium*, *Zuciella caspica* (Middle Asia territories of the former USSR), *Araneus diadematus* (Caucasus). The venoms of these spiders blocked micro-excitabile post-synaptic potentials (mEPSP) and excitabile post-synaptic potentials (EPSP) in locust neuromuscular junctions and potential in a final plate of the frog's muscle. The effect of that venom was irreversible, and during second application, the response was possible to restore after the antagonist was removed by “washing” (physiological solution) of the membrane subjected to the effect of the antagonist [84].

The blockers with similar properties have been found in New Zealand's *Araneidae* venoms: *Argiope trifasciata*, *A. aurantia*, *A. florida*, *Araneus gemma*, *Araneus andreusi* and others [12, 28, 35]. The biologically active fractions of these venoms blocked in the reversible manner the ion currents appearing in the post-synaptic membrane of locust nerve-muscular junction after the application of glutamate [8]. The lower limits of toxins concentration that blocked glutamate-activated potentials in locusts muscle were  $10^{-8}$ – $10^{-9}$  M in the experiments with glutamate ionophoretic application. For obtaining the same blocking effect in experiments with the excitation of nerve terminals, it was necessary to use an order or even higher concentrations of toxins. That fact evidenced that toxin interacts with glutamate channel-receptor complex (CRC) in the activated state, since during the glutamate application, most CRC stay in this state [8]. After washing of the toxin with a solution containing glutamate, a recovery of responses was delayed or not registered at all [8]. However, although a sensitivity of locust post-synaptic membrane was possible to recover by washing of the antagonist with normal physiological solution, there was an irreversibility of toxins' action. However, at the repeated application of toxins to the same preparation, a recovery of the membrane's sensitivity worsened [28].

*Araneidae* toxins affected a kinetics of excitatory postsynaptic currents. Under the influence of toxins, the time of amplitude growth decreased, but their time of amplitude falling often increased and became a biphasic one [8]. The effect of toxins depended on the potential and concentration of substances, and it depended on these variables in the experiments with arginine. For example, the blocking velocity was slowed by reducing of toxins' concentration [8, 28].

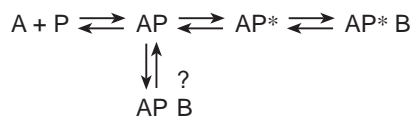
During a study of the composition of venoms' active fractions of *Araneidae* spiders, it was found that some of these toxins are present in different venoms. For example, the venoms of *A. trifasciata*, *A. florida*, *A. aurantia*, *A. andreusi*, *A. gemma* contain the polypeptide with molecular weight of 636 Da, called argiotoxin<sub>636</sub>. Its chemical structure was shown to be identical to the argiopin (AR) previously isolated from a venom of *A. lobata* by Dr. E.V. Grishin [28, 35]. A study of toxins from *N. clavata*, *A. lobata* was pioneering, and a study of antagonists from other *Arthropodae* venoms is in progress [2, 22, 23, 36].

The blocking properties of the argiotoxin<sub>636</sub> were similar to such properties of the argiopin. The results of studying of glutamate-activated single channels in locusts [28, 35] and synaptic transmission in rats' hippocampal slices [6] demonstrated that argiotoxin blocked the glutamate receptors in the post-synaptic membranes in a reversible manner. The argiotoxin was used in concentrations of  $10^{-8}$ – $10^{-9}$  M. The effect of the toxin was dose-dependent at concentrations of  $10^{-4}$ – $10^{-9}$  M and  $2.5 \cdot 10^{-8}$ – $7.5 \cdot 10^{-9}$  M for two series of experiments. In those experiments, the argiotoxin blocked both kainate- and quisqualate-sensitive receptors. In slices of rat's hippocampus, the argiotoxin did not influence GABA-induced inhibition or aspartate-caused spikes which proved that argiotoxin does not interact with GABA or NMDA receptors [6].

There are numerous data on blocking properties of the argiotoxin in experiments aimed at study of single glutamate-activated channels. During a study of kinetics of toxin's action, three types of channels were registered [35]. The first type was characterized by a decrease of probability and frequency of channels opening, an increase of time of their life in a closed state, and practically unchanged (comparing to control) time of life in the open state. For a second type of channels, a further decrease of probability and frequency of channels' opening was registered, a further increase in time of life in

a closed state, and a reducing of the life time in an open state. For the third type, channels opening almost did not happen. With an increase of toxin's concentration or an increase of an exposure with constant concentration, the types of channels could be arranged in the following manner: type 1 → type2 → type 3.

It was demonstrated that similar to the argiopin, the argiotoxin interacts mainly with open channels [35]. However, it can also be bound with closed channel, and this initiate a described sequence of processes. After binding with closed channel, the toxin allosterically positively modulates binding of the glutamate with the complex. That increased a probability of channel opening and, thus, its subsequent blockage by a second argiotoxin molecule. The following blocking scheme was proposed by Jackson [28]:



where A is an activating molecule of the agonist, P,P\* are the receptors that correlated with closed and open state of the channel, and B is a molecule of the antagonist.

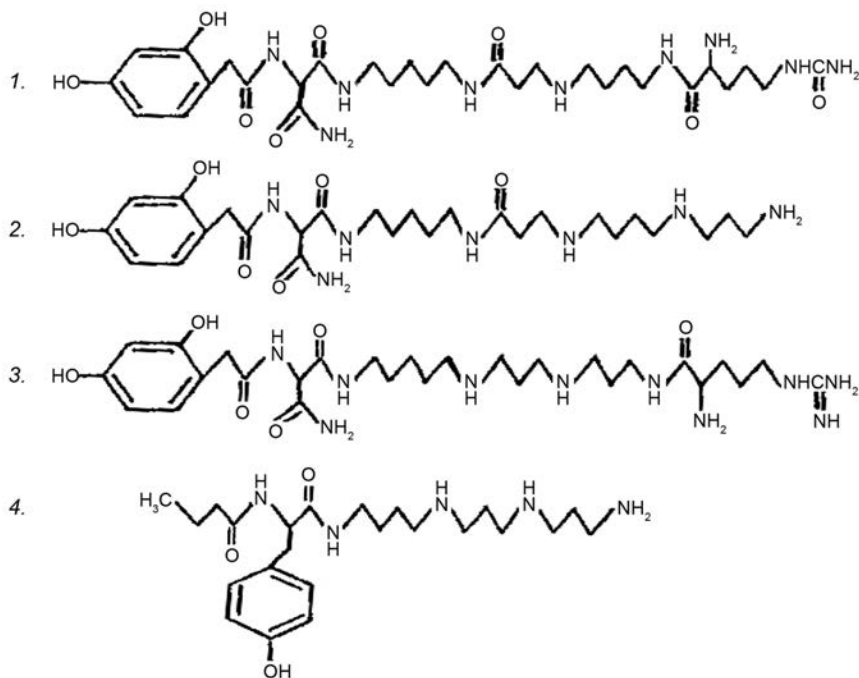
Such scheme of blocking was confirmed by speculations that have been done on a basis of data obtained in the experiments on blocking of single channels. A probability of the effect of toxin binding to the receptor with a site of the glutamate binding is enough low. However, more realistic is a scenario in which the toxin interacts first on the surface of the membrane with lipids or glycocalyx, possibly, being accumulated, and then influences the receptor [28].

There is a high amount of free glutamate found in *Araneidae* venoms, that can reach concentrations of 130–425 mM [17]. This glutamate might play a role in the activation of those glutamates CRC that were inactive previously followed by interaction with the argiotoxin [17].

**Components of venom of *Philanthus triangulum* – antagonists of glutamate receptors.** *Araneidae* are not unique for *Arthropodae* species that are producers of toxins-antagonists of the glutamate receptors. The phylantotoxins from venom of the predatory *P. Triangulum* insect possess similar properties as other known antagonists of the glutamate receptors [35, 77, 96]. Four toxins – alpha, beta, gamma, and delta-phylanto-toxins (PTX) [37] have been purified from the venom of *Philanthus triangulum* and identified; then, the fifth one was detected [8]. Chemical structure of one of them was studied that is the α-phylantotoxin with a molecular weight of 433 Da that interacts with the glutamate receptors (Fig. 1) [28]. Its molecule has common features with spider toxins described above. It is a polyamine coupled with the oxyphenol.

A similarity with *Araneidae* toxins has been also found in the action of PTX toxins toward the glutamate receptors. Delta-PTX (molecular weight of about 700 Da) blocked postsynaptic excitatory currents and glutamate-induced depolarization in the neuromuscular junction of the locust at concentrations of  $10^{-6}$ – $10^{-7}$  M [8]. Besides, it reduced an amplitude and reduced the time of miniature potentials decrease recorded extracellularly [35]. A study of single channels in the locusts muscle showed that delta-PTX blocked glutamate channel-receptor complexes in the activated state. Blocking both integral venom and delta- PTX had a reversible effect. However, unlike the argiotoxin, a recovery of responses was faster if there was an agonist in the washing solution and,

thus, the glutamate receptors were activated [8]. The delta-PTX also demonstrated a presynaptic action by inhibiting of the re-uptake of glutamate [37]. That effect was similar to the action of JSTX in the experiments with glutamate re-uptake by rats' synaptosomes [75, 76].



**Fig. 1.** Chemical structure of toxins of the arthropods: 1 – JSTX-3; 2 – NSTX-3; 3 – argiopin AR; 4 – PTX 433 [23, 40]

**Рис. 1.** Хімічні структури токсинів членистоногих: 1 – JSTX-3; 2 – NSTX-3; 3 – аргіопін AR; 4 – PTX 433 [23, 40]

The polyamine beta-PTX toxin with a molecular weight of 243 Da was also studied. This toxin inhibited glutamate postsynaptic potentials in the nerve-muscular junctions of the locusts, but its effect was weaker than that of delta-PTX [37]. The action of beta-PTX was a reverse one: the amplitude of the EPSP was restored when the toxin was washed out by the physiological solution. A degree of blockage depended on the concentration of beta-PTX. During electrical stimulation of the neuromuscular preparation, the effect depended on frequency of stimulation: in the interval of 5–20 s between impulses, the effect of blocking increased with an increase of frequency. These experiments, as well as experiments using concanavalin A, demonstrated that beta-PTX influenced desensitization of the glutamate receptor and increased a duration of desensitization period [37]. Experiments conducted on single channels have shown that beta-PTX binds to the activated glutamate receptor [37].

It should be noted that the insufficiently well-purified preparation of *P. triangulum* venom caused a weak depolarization of the post-synaptic membrane in the nerve-muscular junctions of the locusts [37].



**Study of molecular structures of glutamate receptors' antagonists obtained from *Araneidae* venoms.** This issue has been briefly described in our previous studies [2, 36, 38–57]. Chemical, biochemical and electrophysiological characterization of the venoms and toxins from the Arthropods have been also carried out. An improvement of the methods of isolation of the individual chemical substances from the venoms permitted in 1990th to define the molecular weight and chemical structure of some toxins. Until 1988, three most important for practical use and best studied *Araneidae* toxins were obtained. They are JSTX-3, NSTX-3, and argiopin.

*Methods of purification of venoms and toxins from *Nephila* spiders.* In 1986, the venoms were isolated from *N. clavata* and *N. maculata* and used for purification of toxins [5]. The venomous spiders' glands were homogenized in 60% acetonitrile and the homogenate was centrifuged for 5 min at 3,000 rpm. The supernatant was subjected to a reverse phase high-performance liquid chromatography (HPLC) using the octadecylsilane column (Toyo Sods, ODS TSK-120T, 4.6×250 mM) in 0.02% HCl and acetonitrile gradient concentration 0–50% during 30 min with elution rate of 0.5 mL/min. The venom from *N. clavata* contained four components possessing toxic properties – JSTX-1, JSTX-2, JSTX-3, and JSTX-4. The venom of *N. maculata* contained three such components – NSTX-1, NSTX-2, NSTX-3. JSTX-3 toxin was the main biologically active element of *N. clavata* venom, and its concentration in the venom was the highest [5]. The chemical formula of this toxin is presented in Fig. 1. Its molecule consists of the 2,4-dihydroxyphenylacetate-asparagine coupled to asparagine, and through it to the polyamine [5, 28]. In the JSTX-4 molecule, the argiopin is coupled to JSTX-2 with a peptide link (Fig. 2, 3) [5]. Separate toxic components differ between themselves by their polyamines' structure (see Table):

#### Toxins from *N. clavata* and their chemical structure

##### Токсини з *N. clavata* і їхня хімічна структура

Chemical structure	Toxin
- (CH <sub>2</sub> ) <sub>3</sub> NH (CH <sub>2</sub> ) <sub>3</sub> NH <sub>2</sub>	JSTX-3
- (CH <sub>2</sub> ) <sub>3</sub> NH (CH <sub>2</sub> ) <sub>4</sub> NH	JSTX-2
- (CH <sub>2</sub> ) <sub>3</sub> NH (CH <sub>2</sub> ) <sub>4</sub> NH - Arg	JSTX-4

NSTX-3 is the main active agent in *N. maculata* venom (Fig. 1). It is 2,4-dihydroxyphenylacetate-asparagine (arginylcadaverno-beta-alanyl) cadaverin that is very similar to JSTX-3 (Fig. 1). According to mass spectroscopic investigations, its molecular weight is 665 Da [5]. *N. clavata* venoms slightly differ in their physiological action of various fractions [70, 71]. Effects of some of them were reversible, while the others, for example, JSTX-3 acts irreversibly. Poorly purified JSTX-3 were stored in frozen state since they changed their properties unpredictably. At the beginning, they acted irreversibly, then with time, in electrophysiological experiments it was possible to remove its molecules from the cell membranes [70, 71].

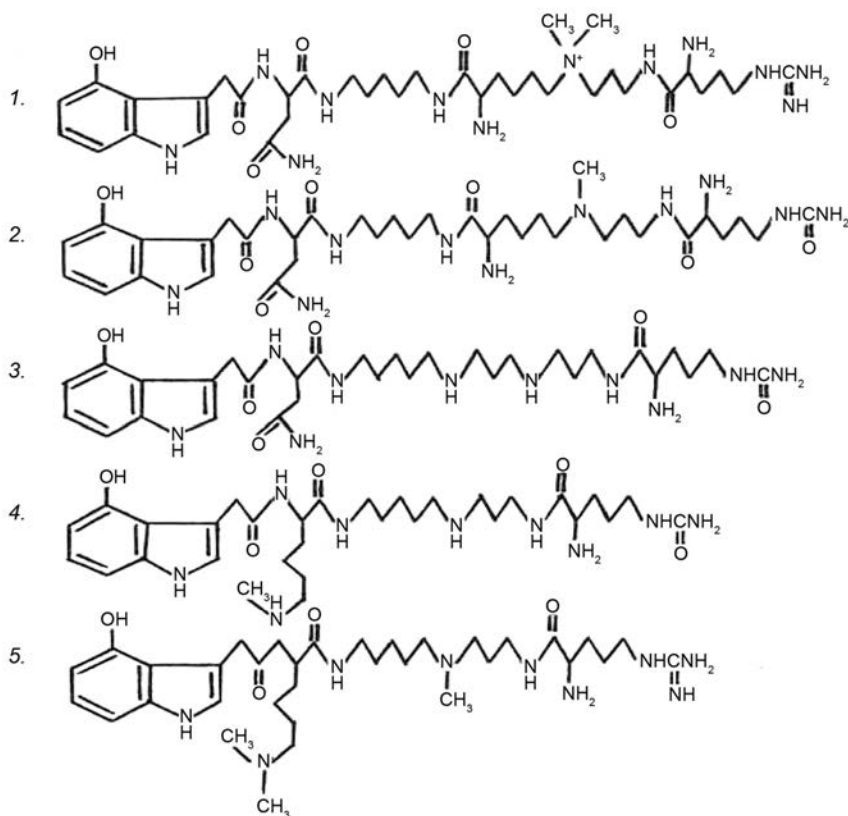
In 1987, JSTX-3 toxin and four its analogs were synthesized in Japan. The analogs had different polyamines' structures, and all of them blocked the glutamate receptors [26]. In 1987, NSTX-3 toxin was also synthesized [87].

*Study of chemical structure of the toxins obtained from *A. lobata*.* The argiopin is the main active component of *A. lobata* venom (Fig. 1), and the concentration of AR was the highest in this venom [12]. Ability of *A. lobata* venom, mostly the argiopin, to block

the glutamate receptors were determined [61, 62]. T. Budd et al. [12] showed that the argiopin is the main active component of *A. lobata* venom, and its concentration there was the highest. The molecular weight of AR in the obtained active substance was 646 Da. Its chemical structure was defined (Fig. 1). It is a 2,4-dihydroxyphenylacetate-asparagine that is linked through a polyamine with the terminal arginine.

There are publications devoted to the isolation of fractions of the *A. lobata* venom containing toxins of different types of actions. E.V. Grishin et al. elaborated the method of obtaining the toxin from that venom [22, 23]. 0.3% toxin was sedimented from a water solution using 60% ethanol. That sediment was separated by centrifuging during 10 min at 5,000 rpm. The supernatant was concentrated and analyzed using a reverse phase HPLC on the column (22.7×250 mm, Zorbax C<sub>8</sub>) in 0.1% trifluoroacetic acid in acetonitrile gradient (0–15%) during 75 min with the elution rate of 10 mL/min. The molecular weight of purified toxin obtained using this method was near 1% of initial weight of the venom; all other venom fractions (substances) were removed [22, 23].

In addition to the argiopin, at least nine compounds have been isolated from *A. lobata* venom (Fig. 2), and their biological activity was found to be similar to that of the argiopin. Like the argiopin, they were isolated by the HPLC [22, 23], but from other fractions of the venom (Fig. 3). The argiopin was detected in fraction 1. Fractions 3, 7 – 0 were

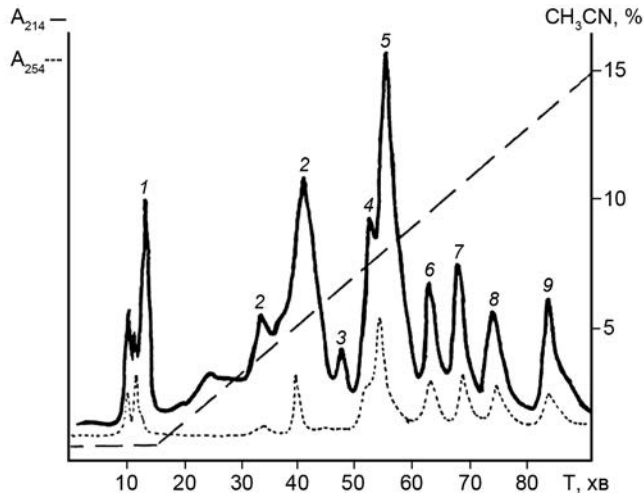


**Fig. 2.** Chemical structure of toxins from the argiopinins' family – ARN [23, 40]

**Рис. 2.** Хімічна структура токсинів із родини аргіопінінів – АРН [23, 40]

shown to be the individual compounds. Other components were isolated using a re-chromatography of the fractions 4–6 and 10 using a column with a reverse phase in similar conditions. Further, chemical structures of all isolated toxins were studied in details [22, 23]. These substances are homologous according to their chemical structure and contain the chromophore group in their molecule (phenol or indole, asparagine (with a few exceptions), and polyamines of different lengths). According to the results of UV-spectroscopy, all components of the venom were separated into three types: argiopin, argiopinins and pseudoargiopinins (Fig. 1, 2). Thus, argiopin contains phenolacetic acid, argiopinins – dihydroxyindolacetic acid, and pseudoaryhopinins – indolacetic acid. In some compounds, the asparagine may be replaced by a modified lysine (Fig. 1, 2). The aliphatic polyamines whose length and structure are different for specific compounds, is a necessary component of each toxin. Finally, each of these substances, except the pseudoargiopin-3, contains the arginine or its residue with a free  $\alpha$ -amino group [22, 23].

In 1986, E.A. Elin et al. obtained a synthetic argiopin. Such synthetic argiopin also possessed an ability to block the glutamate receptors [18].



**Fig. 3.** Profile of separation of components of *A. lobata* venom [22, 23]. The separation was conducted by means of HPLC on the column (22.5×250 mm) filled with TSK ODS-120T in 0.1% trifluoroacetic acid in a gradient of acetonitrile concentration with the elution rate of 10 ml/min

**Рис. 3.** Профіль фракціонування компонентів отрути *A. lobata* [22, 23]. Розділення виконували за допомогою вискоєфективної рідинної хроматографії (верх) на колонці (22,5×250 мм), наповненій TSK ODS-120T, у 0,1% трифтороцтовій кислоті у градієнті концентрації ацетонітрилу за швидкості елюції 10 мл/хв

### Components of venoms obtained from other spiders – *Argiope* and *Araneus*.

It was demonstrated that active fractions isolated from the venoms of spiders-Arachnids of American continent were similar from the point of view of their physiological properties. Besides, their action was similar to the action of active fractions of *A. lobata* toward the glutamatergic synapses [12, 28]. The composition of the venoms from *A. trifasciata*, *A. gemma*, *A. florida* was studied. In general, methods of isolation and purification of toxins from these spiders' venoms are similar to methods developed previously for other



toxins [5, 22, 23]. Isolation was carried out using a reverse phase HPLC [12]. Then the active fraction was studied after the re-chromatography on the  $C_{18}$  column with the elution rate of 2 ml/min in 0.08% solution of trifluoroacetic acid (5 min), and then in the acetonitrile gradient for 15 min in 4% solution and during next 10 min – in its 10% solution.

A number of active fractions was obtained and purified from the venoms of the abovementioned *Araneidae*. The results of studying chemical structure of toxins from such *Araneidae* venoms demonstrated that they contained the same substance named the argioxin, whose molecular weight was 636 Da. According to the results of the UV spectroscopy, their chemical structure is the same as structure of the argiopins. It was proved that active fraction of the venoms of numerous *Araneidae* is the argiopin, or the argioxin<sub>636</sub> [12, 28].

In addition to argioxin<sub>636</sub>, other toxins have been obtained from the venoms of *A. trifasciata* and *A. florida*. Their chemical structures are homologous and might be sub-divided into two families. The first family contains the phenolic derivatives, and this family of toxins includes the argioxin<sub>636</sub> and its methylene derivatives with a molecular weight of 622, 650, 480, 466, 637, 678, 720 Da. While the second family of toxins includes the indolyl derivatives of similar structure having another chromophore group. In *A. trifasciata*, the main active agent is not the argioxin<sub>636</sub>, and principal toxin of this family is a peptide with a molecular weight of 659 Da. Its homologues have the molecular weight of 673, 645, 503, 517 Da, respectively [12].

A contribution of each of these substances to total toxic effect of venoms is different. According to the biological activity, all substances within this family can be arranged in the following order (noted in the molecular weight) [12]:

1st family: 636 → 622 → 650 → 480 → 466 → 637 → 678 → 720

2nd family: 659 → 673 → 503 → 517

The venoms of various spiders' species differ according to a quantitative composition of their components. For example, in *A. florida*, concentration of the argioxin<sub>673</sub> is the highest among toxins of the 2nd family [28]. The argiopin<sub>659</sub> (2nd family) according to the described properties coincides with the toxin which Jackson isolated from *A. aurantia* that irreversibly blocked a transmission between the cochlear nerve and the cochlear nucleus of birds [28].

As one can see from Fig. 1 and 2, chemical structure of the antagonists of the glutamatergic synapses isolated from the venoms of various *Araneidae* have many common features. All of them contain: 1 – aromatic group (phenolacetic or indolacetic), 2 – asparagine or modified lysine, 3 – aliphatic polyamine of different lengths, 4 – many of them have N-terminal arginine [5, 12, 22, 23, 28].

Abovementioned toxins form two families depending on the aromatic groups which they contain. The family of the phenolacetic acid derivatives contains the most widespread and investigated toxins – the argiopin (argioxin<sub>636</sub>), JSTX-3, NSTX-3 [28], while the family of the indolylacetic acid derivatives is less studied and contains a number of toxins from *A. lobata* (argiopinins, pseudoargiopinins), *A. gemma*, *A. trifasciata* and other *Araneidae* [12, 22, 23].

Organisms of *Araneidae* contain the same set of toxic substances as their venoms. Difference between these venoms from various species is the absence or presence of separate components, as well as a quantitative correlation of these components. Based on these differences in chemical composition, various venoms possess different physiological properties [12].

**Role of different sites of toxins' molecules in blocking the glutamate receptors.** A contribution of various sites of toxin molecules into the effect of blocking the glutamate receptors is different. The role of each of these fragments and mechanisms of their interaction with the receptor are the points of interest. Most researchers suppose that the aromatic groups (residues of phenol- or indolacetic acids) play the main role in the interaction of toxins with the receptors, because these fragments are obvious part of each toxin. There are results in favor of this point of view [75, 76]. The effects of JSTX-3 and its two fragments – 2,4-dihydroxyphenylacetate-asparagine (DHPPhA-Asp) and 2,4-dihydroxyphenylacetate-asparagine (DHPPhA) – on binding of marked glutamate to synaptic membranes of rat brain were investigated. All three compounds blocked the binding in a dose-dependent manner. Their effects differed in that the first two substances demonstrate the same effect in approximately the same quantities, and the DHPPhA was necessary about 3–5 times more for this. A complete inhibition of binding was reached at 0.33 mM DHPPhA. The 2.5 DHFU and 3.4 DHFU isomers also suppressed binding of the glutamate. In the control experiments, L-glutamate was substituted effectively with diethyl ether of glutamic acid, a glutamate receptor antagonist [75, 76]. *Araneidae* venoms contain DHPPhA in its free state, and it is possible that in natural conditions these molecules also participate in blocking the receptors [17].

In other studies of these authors, a difference in properties of JSTX-3 and DHPPhA-Asp was registered [76]. It was found that JSTX-3 and DHPPhA-Asp act in different way on sodium-dependent and sodium-independent seizure of glutamate by the synaptosomes of the rat brain membrane. Equal concentrations of JSTX-3 and DHPPhA-Asp depressed in equal rates sodium-independent binding of glutamate (for different concentrations of the toxins). DHPPhA-Asp was significantly less effective than JSTX-3 in blocking of sodium-dependent binding: to achieve the same level of blockage, the DHPPhA-Asp concentration should be 10–15 times higher than concentration of the JSTX-3.

The role of polyamine in the effects of toxins was demonstrated in experiments on blocking of the excitable postsynaptic potential (EPSP) by various JSTX-3 analogues [26]. All analogues contained DHPPhA-Asp combined with the polyamines of different length, but they were shorter than a one in the JSTX-3. The extension of the polyamine chain was followed by a formation of more stable toxin-receptor complex, and if, under the action of analogue with the shortest chain, the amplitude of the EPSP was restored with compound removing, then the action of JSTX-3 became practically the irreversible.

When properties of the argioxin<sub>636</sub> analogues (argiopin) were studied, the role of N-terminal arginine in blocking was demonstrated. The modification of the end of this molecule led to a complete loss of its activity [12].

The RTX<sub>433</sub> molecule from *P. triangulum* toxin is very similar to *Araneidae* toxins. It also consists of oxyphenol connected with the polyamine. However, its polyamine does not contain asparagine or arginine. The RTX<sub>433</sub> molecule is an effective blocker of the glutamate receptors with properties that are close to properties of *Araneidae* toxins. Thus, it might be expected that phylantotoxins can be also used for studying the glutamate receptor along with families of toxins from *Araneidae* [28].

A number of publications were devoted to a study of the molecular mechanisms of toxins' interaction of with the receptors. A protein named GBP was obtained from synaptic membranes that was able to bind glutamate [67, 68]. It was demonstrated that the

glutamate binding to this protein was inhibited by the reagents of the FeS groups –  $\text{NaN}_3$ , KCN, chelating agents like the *o*-phenanthroline. Therefore, it was suggested that the membrane glutamate binding protein is a glycoproteide with FeS group at the site of ligand binding, and the spider toxins *A. gemma*, *A. aurantia*, *N. arabesca* acted on the GBP in the same way as the reagents acted on the FeS groups. This may evidence that toxins interact with the same prosthetic group [67, 68].

Subsequently, a qualitative analysis of the reaction of toxins binding with the FeS groups was carried out. The reagent on the  $\text{FeCl}_3$  phenols that contacts with it gives a dense green color of the solution [82]. The same change in color appeared when  $\text{FeCl}_3$  was added to JSTX-3 solutions ( $\lambda_{\text{max}} = 630 \text{ nm}$ ), and it disappeared when EDTA was added [97]. No other metal ions gave such coloration at contact with phenols or JSTX-3 – it was much lighter in reactions with  $\text{Hg}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Ce}^{3+}$ ,  $\text{Ce}^{4+}$ . An existence of such specific reaction between the  $\text{Fe}^{3+}$  ions and JSTX-3 permitted an assumption that in a result of their interaction appears the chelating complex with development of green color of solution [97]. A schematic representation of such complex is shown on Fig. 4.

As a result of those studies, it was concluded that *Araneidae* toxins, and JSTX-3, in particular, can form the chelating complexes with the prosthetic group of glutamate membrane receptors. This mechanism of toxins interaction with the glutamate receptors was confirmed in the experiments on the neuromuscular junction of lobster. The blocking characteristics of JSTX-3 significantly deteriorated with the addition of  $\text{Fe}^{3+}$  ions in the culture medium [74, 75, 97].

Thus, the results presented in our review demonstrated that the arthropods' toxins, both phenol and indole derivatives, may be useful in the laboratory experiments. The molecular weights and chemical structure of the most important toxins were studied until the end of 20th century [2, 36, 38–57]. Of practical significance might be three *Araneidae* toxins synthesized at that period: JSTX-3, NSTX-3, and the argiopin. Synthesis of JSTX-3 toxin and its four analogs with different polyamines' was conducted, and they all blocked the glutamate receptors [26]. A synthetic argiopin also possessed an ability to block the glutamate receptors [18].

Chemical structure of antagonists of the glutamatergic synapses isolated from the venoms of various *Araneidae* has several common features. All these aganists contain: 1 – aromatic group (phenolacetic or indolacetic), 2 – asparagine or modified lysine, 3 – aliphatic polyamine of different lengths, 4 – many of them have N-terminal arginine [5, 12, 22, 23, 28].

The abovementioned toxins form two families depending on the aromatic groups which they contain. A family of phenolacetic acid derivatives contains the most widespread and investigated toxins – the argiopin (argioxin<sub>636</sub>), JSTX-3, NSTX-3 [28]. The

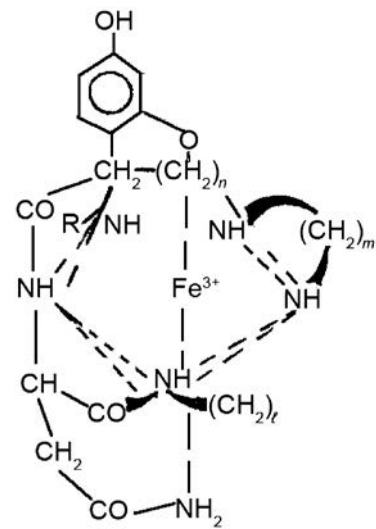
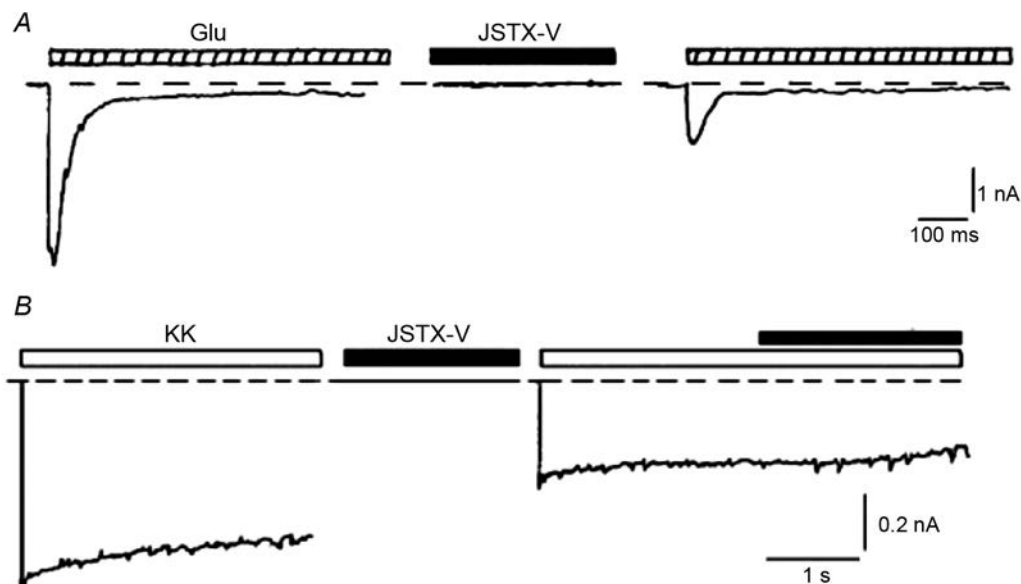


Fig. 4. Hypothetical structure of the chelating complex of JSTX-3 with cation  $\text{Fe}^{3+}$  [97]

Рис. 4. Гіпотетична структура хелатного комплексу JSTX-3 з катіоном заліза  $\text{Fe}^{3+}$  [97]

family of the indolylacetic acid derivatives is less studied and contains a number of toxins obtained from *A. lobata* (argiopinins, pseudoargiopinins), *A. gemma*, *A. trifasciata* and other *Araneidae* [12, 22, 23].

A study of toxins from *N. clavata* and *A. lobata* was carried out in Ukraine by a scientific team under a supervision of O.A. Krishtal [2, 36] (Fig. 5). As a result of their studies, they concluded that the 2,4-dihydroxyphenylacetate-asparagine fragments play a key role in toxin interaction with the glutamate CRC. These fragments are similar in all these antagonists and define their biological activity. The length and structure of polyamines define the individual differences in toxins' properties.



**Fig. 5.** The venom JSTX-V blocks chemo-activated transmembrane electrical currents in membrane of rat hippocampal pyramidal neuron: *A* – influence on glutamate-activated (Glu) currents; *B* – influence on kainate-activated (KK) currents. After the registration of the control responses to Glu and KK, the membrane was maintained in Ringer solution with JSTX-V ( $2.5 \times 10^{-4}$  units/ $\mu$ l) during 3 min. In this experiment, the amplitudes of chemo-activated currents decreased after the JSTX-V influence. Experiment with JSTX-V re-application in the same concentration ( $2.5 \times 10^{-4}$  units/ $\mu$ l) on the background of KK is shown on (*B*). Concentrations of Glu and KK were 1 mmol/l. V hold – 100 mV [42]

**Рис. 5.** Отрута JSTX-V блокує хемоактивовані трансмембранні електричні струми у мембранах пірамідних нейронів гіпокампу щура: *A* – вплив на глутамат-активовані (Glu) струми; *B* – вплив на кайнат-активовані (KK) струми. Після реєстрації контрольних відповідей на Glu та KK мембрану витримували у розчині Рінгера з JSTX-V ( $2,5 \times 10^{-4}$  од/мкл) протягом 3 хв. У цьому експерименті амплітуди хемо-активованих струмів зменшувалися після впливу JSTX-V. Експеримент із повторною аплікацією JSTX-V у тій же концентрації ( $2,5 \times 10^{-4}$  од/мкл) на тлі KK показано на (*B*). Концентрації Glu та KK становили 1 ммоль/л. V підтр – 100 мВ [42]

In those studies, it was found that JSTX-3 is a unique toxin among others from the point of view of a correlation between its chemical structure and the physiological effects, and the most simple structure is defining maximal effect [8]. The argiopin toxin also possesses unique properties among the toxins obtained from *A. lobata*, because of optimal correlation between its chemical structure and physiological effect [22, 23]. As a result of these studies, it was concluded that *Araneidae* toxins, and JSTX-3 in particular, can form

the chelating complexes with the prosthetic group of the glutamate receptors in plasma membrane. This mechanism of toxins interaction with the glutamate receptors was studied also in experiments on a neuromuscular junction of the lobster. The blocking characteristics of JSTX-3 significantly deteriorated with the addition of  $\text{Fe}^{3+}$  ions to the cultural mixture [74, 75, 82, 97]. Physiologically active toxic molecules with such chemical structures were also suggested to be used in biotechnology [41, 42].

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## БИОЛОГІЧНОАКТИВНІ ПОХІДНІ ФЕНОЛУ Й ІНДОЛУ НАЗЕМНИХ ЧЛЕНИСТОНОГИХ: ЕЛЕКТРОФІЗІОЛОГІЧНІ ТА ХІМІЧНІ ВЛАСТИВОСТІ

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Токсини-антагоністи глутаматергічних синапсів були виявлені в отрутах різних наземних членистоногих – як комах, так і павуків. Відома хімічна структура деяких із них, більшість є похідними фенолу й індолу. Їх успішно застосовують для електрофізіологічних досліджень мембранних структур, і цей огляд присвячено деяким результатам досліджень таких речовин, що можуть бути корисними для лабораторної практики. У публікації описано експериментальні дослідження хімічних структур паралельно із результатами електрофізіологічної реєстрації ефектів, які спричиняють отрути і токсини деяких членистоногих. Запропоновано пояснення фізіологічної дії речовин-антагоністів глутаматних рецепторів залежно від особливостей їхньої хімічної будови. Обговорено роль різних фрагментів молекул токсинів у блокуванні глутаматних рецепторів. Також наведено інформацію щодо компонентів отрут комах родин *Sphecidae* (*Phlanthus triangulum*) і *Araneidae*, а саме кількох видів павуків із родів *Argiopo*, *Araneus*, *Nephila*.

**Ключові слова:** отрути членистоногих, токсини, антагоністи рецепторів, трансмембранний електричний струм

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