VENOM AND TOXINS FROM ARGIOPE LOBATA: ELECTROPHYSIOLOGICAL STUDIES

O. M. Klyuchko

National Aviation University, 1, Lubomyr Huzar Ave., Kyiv 03058, Ukraine
e-mail: kelenaXX@nau.edu.ua

Klyuchko O.M. Venom and toxins from Argiope lobata: electrophysiological studies. Studia Biologica, 2020: 14(2); 39–56 • DOI: https://doi.org/10.30970/sbi.1402.618

The purpose of this study was to present experimental data on the action of venom and toxins from Argiope lobata spiders on the glutamate channel-receptor complex. Kainate was used as a glutamate channel-receptor complex agonist because it initiated non-inactivated inward ionic transmembrane electric currents in rat hippocampal membranes. Effects of antagonists can be studied on the background of such currents. Chemo-activated currents and glutamate channel-receptor complex antagonists from A. lobata were studied. Electrophysiological experiments were performed and all chemicals were applied to perfused hippocampal pyramidal neuronal membranes using the ‘concentration-clamp’ technique. A conventional electronic circuit was used for single-electrode voltage-clamp recording. All substances under study – integral venom, argiopin, argiopinine 1, argiopinine 2 – demonstrated similar properties. The amplitudes of ionic currents activated by glutamate, kainate and quisqualate decreased after the application of these antagonists to the rat hippocampal membrane under the voltage-clamp conditions. The kinetics of currents’ activation and desensitization (in case of glutamate and quisqualate) were not affected by the antagonists. The effects of argiopin and integral venom were investigated within the concentrations of 5·10^{-8}–1·10^{-2} mol/L and 10^{-4} g/mL, respectively. At these concentrations, neither integral venom, nor argiopin suppressed glutamate-, kainate-, quisqualate-activated currents completely. The amplitude of non-blocked integral venom components averaged 14.4% of the original value for kainate-activated currents. Argiopin reduced the amplitudes of kainate-activated currents to 19% of the control values. Argiopinine 1 and argiopinine 2 acted in a very similar way. Both substances caused reducing of glutamate- and kainate-activated ion currents amplitudes acting in small quantities of 10^{-5}–10^{-6} mol/L. The differences between them were in the quantitative characteristics of the blocking action. Such effects as “dose–effect” dependency, the antagonists’ influences on activated and inactivated receptor;
kinetics of the antagonists' action and their removal, analysis of dissociation constants were studied under the antagonists' influence. Conclusions about the mechanisms of the antagonists' influence on glutamate channel-receptor complex, as well as a comparison of the caused effects were made.

**Keywords:** Araneidae, venom, toxin, glutamate receptor antagonist, transmembrane electric current

**INTRODUCTION**

In neurons of the mammalian brain, according to the classical concepts, two mechanisms of information transmission coexist – through electrical impulses and through the activation of electrical processes by the agonists – specific chemical substances. Both mechanisms are interrelated, and their research has been reflected in numerous scientific works over the last decades. They formed our modern understanding of physical and chemical mechanisms of brain functioning [6, 8–13, 17, 20, 28, 29, 31]. In this article, the experimental data of our researches of glutamate (GLU) receptors' agonist kainate (KK)–sodium salt of kainic acid, which initiate inward ion transmembrane electric currents in rat hippocampal membranes, and the action of venom and toxins from *A. lobata* spiders on them are presented. Arthropodae toxins as research tools in electrophysiology became extremely popular in the works of contemporary researchers [4, 6, 7, 9, 15, 18, 25–32].

The results of our studies of toxins – glutamate channel-receptor complex (gCRC) antagonists were suggested. Among them, there are such gCRC antagonists from *A. lobata* spiders as integral venom AR-V, its main active component toxin argiopin (AR), as well as two other toxins of corresponding homologous series: argiopinine 1 (ARN-1), argiopinine 2 ARN-2 [20]. Chemical structures of these compounds – phenol derivative AR, and indole derivatives ARN-1, ARN-2 were presented [20, 21]. In some of those works, the results of studying of chemical structures of Araneidae toxins, as well as the caused electrophysiological effects, have been described [1–3, 5, 12, 13, 16, 22–32].

Investigation of *A. lobata* toxins (as well as other Araneidae toxins) is rather important because of high specificity of these substances and the effectiveness of their action. For the first time, the results of electrophysiological investigation of chemosensitive transmembrane currents in brain neurons influenced by *A. lobata* venom and three toxins isolated from it have been published in comparison. The effects of the less known toxins ARN-1, ARN-2 have been demonstrated in more details. However, such toxins became known not only due to their traditional role as tools for neurophysiological investigations. All toxins of this type are amphiphilic substances, phenol and indole derivatives with polyamine chains of different lengths and complexity, and they have extremely interesting properties. Nowadays, they are considered to be very promising for application in various fields of practice including pharmacology [8], biotechnology [19, 21–23], nanotechnology [21], as well as agriculture for protection against the pests [10, 24]. They were used in the newly developed methods [19, 23], and for some of such results, patents were obtained [19, 22–23]. Previously, it was suggested that such toxins could be used as a universal “marker” of glutamate receptors in different representatives of fauna (like JSTX). So, a significant similarity of glutamate receptors in different phylogenetically distant species was supposed. Because of the importance of the results of Araneidae venoms and toxins study and their applications, our previous reviews
were devoted to fundamental works of the authors who studied such substances [4, 6, 7, 9, 15, 18–32]. The results of studies of Araneidae toxins chemical structures, as well as electrophysiological effects they cause blocking different channel-receptors complexes (CRC) are in [12, 13, 20, 21].

The aim of this publication was to present experimental data about the action of venom and toxins from *Argiope lobata* spiders on glutamate channel-receptor complex.

**MATERIALS AND METHODS**

*Electrophysiological study of the action of A. lobata toxins.* Chemo-activated transmembrane currents, as well as the action of gCRC antagonists from *A. lobata* were investigated. The development of experimental methods was based on the methods previously elaborated by scientific groups under the guidance of members of the USSR Academy of Sciences and NAS of Ukraine P. G. Kostyuk and O. O. Krishtal, and by the research group including Dr. A. Ya. Tsyndrenko, Dr. M. I. Kiskin, Dr. O. M. Klyuchko. A brief description of experimental studies of GLU- and KK-activated currents was presented previously [1, 12, 13, 22]. Our experiments were conducted on internally perfused rat hippocampal pyramidal neurons of 7- to 15-day-old rats. All manipulations with animals were carried out in accordance with the International Convention of animals and the Law of Ukraine “On protection of animals from cruelty”. Protocol N 2 (October 20, 2016) of the Bioethics Committee of the Educational and Scientific Centre “Institute of Biology and Medicine” of Taras Shevchenko Kyiv National University. Hippocampal slices were treated enzymatically with a subsequent mechanical isolation of single pyramidal neurons [19, 22–23]. A microphotograph of the isolated pyramidal neurons is shown in Fig. 1A. Experiments were carried out using solutions described below as solutions A, B, C, D. These solutions were considered the basic ones and their contents were changed depending on the requirements of experiments (more detailed information about solutions’ preparations and use see below). All experiments were performed at 20 °C (21–23 °C). All chemicals were applied to internally perfused hippocampal pyramidal neurons, using the ’concentration-clamp’ technique (Fig. 1A, B) [1]. This technique provides convenient tools for examining of chemo-activated currents, as the external solution can be changed within a few milliseconds to 20 ms (depending on the preparations) in a step-wise manner [1, 23]. The membrane potential was measured with an Ag-AgCl wire mounted on the patch pipette holder, and the reference electrode employed was an Ag-AgCl wire in the external solution. A conventional electronic circuit was used for single-electrode voltage-clamp recording [1]. Both current and voltage were monitored and the data were simultaneously stored in a computer for detailed offline analysis. When 1 mM L-Glu was applied to an isolated pyramidal neuron, internally perfused and clamped to -80 mV, a transient inward current was elicited [1]. The holding voltage was changed in some experiments to less negative values, but no difference in the investigated phenomena was observed. The experiments were carried out under computer control. The scheme of the experimental setup that was used for electrophysiological study of transmembrane ion currents in voltage-clamp mode is shown on Fig. 1B. Indications on the scheme 1B. 1 – neuron at the pore of glass micropipette. 2 – micropipette was filled with solution A for intracellular perfusion; mobile cassette with experimental chambers with different solutions for the application (B₁, B₂, B₃) in three different chambers; arrow K₁ indicates directions of chambers with these solutions movement. 4 – tube in which cell 1 was moved from one chamber to another; applications of...
substances to the surface of neuronal membrane were done in this tube according to
the following procedures. Cell 1 on the micropipette 2 was inserted into the tube 4
(arrow $K_2$ indicates the direction of movement). When electromagnetic valve 5 was
opened, a quick application of solution $B_2$ was done; this solution was sucked into
the tube due to the negative hydrostatic pressure. The dark arrows indicate the directions
of the solutions flow during their application in tube 4, and flows’ directions in the micro-
pipette 2 during the cell fixation at the pore. The dotted line limits the mechanical part
of experimental setup. 6 – amplifier of holding potential $V_m$ and command $V_{com}$, 7 – device
for measuring the potential. 8 – amplifier of the registered transmembrane current.

**Fig. 1.** The scheme of the electrophysiological experiment on registration of transmembrane electric currents in voltage-clamp mode: $A$ – micrographs of the isolated rat hippocampal neurons (10 μm in 1 cm); $B$ – the scheme of the experimental block

**Solutions and reagents.** The processes of solutions preparation and use, as well as
modes of preparation of cell suspensions and cultures was an important component of
the experiments; the methods were protected by the patents [19, 22, 23]. The details of
their preparation are described below. The following solutions were used in the experi-
ment (all concentrations are given in mmol/L).

**Solution A.** $KF = 100$, Tris-Cl = 30, pH = 7.2. Solution A was used as an intracellular
one and its composition was not changed during the experiments.

**Solution B.** NaCl = 156, MgCl$_2$ = 1.1, Hepes-NaOH = 20, CaCl$_2$ = 2.6, pH = 7.4.
Solution B was used as an extracellular one during the experiments. The following
substances were added to it in the course of the experiments:

a) L – glutamate (GLU), kainate (KK), quisqualate (QL), glycine, γ-aminobutyric
acid (GABA); b) integral venom JSTX-V, its active component toxin JSTX-3; c) integral
venom AR-V, its active component toxin AR as well as other toxins from this venom:
ARN-1, ARN-2; d) other substances.

All toxins from AR-V were obtained using high performance liquid chromatography
(HPLC) at the Shemyakin–Ovchinnikov Institute of Bioorganic Chemistry, the Academy of
Sciences of the Russian Federation, Moscow [11–13]. Their concentration was expressed
in mol/L. Chemical structures of these synthesized compounds were studied [12–13] and
used in the experiments [12, 13, 20]. It is evidenced that the analogues of the studied compounds were found among hydrocarbon technogenic pollutants.

In addition to the above solutions, other ones of the following composition were used in the preparation of the object for the experiment [19, 22, 23]. For rat hippocampal neurons dissociation the following solutions were used (all concentrations are given in mmol/L): solution C – NaCl = 150, KCl = 4, Heps-NaOH = 20, glucose = 10; solution D – NaCl = 150, KCl = 4, NaHCO₃ = 26, CaCl₂ = 0.9, EGTA = 1.0, glucose = 10. In solution D, the concentration of free calcium was 5.0 · 10⁻⁷ mmol/L. This solution was saturated with carbogen (5% CO₂, 95% O₂) to pH = 7.4. Dry Eagle’s Minimal Essential Medium (MEM) and Dulbecco’s Modified Eagle Medium (DMEM) (Serva), as well as poly-L-lysine (Sigma) were used for the cell culture. For enzymatic tissue processing, the complex of proteolytic enzymes from Aspergillus oryzae produced by Chemreactive, Olaine (Latvia) was used, as well as enzymes trypsin PM-14 (Serva), pronase E (Serva), collagenase type IV (Sigma) [19, 22, 23].

*Isolated hippocampal neurons.* New methods for dissociation and cultivation of rat hippocampal neurons were developed [19, 22, 23]. This was due to the fact that selected neurons of high nervous system, namely the rat hippocampal pyramidal neurons, are very “delicate” and may be damaged easily. The majority of experiments were performed on isolated neurons immediately after the enzymatic dissociation. The preference was given to rats of the age under 2–3 weeks because the differentiation of their pyramidal cells ends before this age. Relying on already known methods of cells isolation [1, 22], we have developed new methods aimed at causing the least possible damage to non-NMDA type receptors [19, 23]. After rat decapitation, the hippocampus was taken away and moved to solution C, as described above, in the shortest possible time. We made cross slices of the hippocampus (300–400 µm) by a thin blade. These slices were placed into the solution of enzymes prepared on the basis of solution D. Different enzymes were selected for nerve tissue dissociation, and the following optimal regimes were proposed. Two complexes: of pronase (0.3%) with collagenase (0.1%) (prototype method) and proteolytic enzymes from Aspergillus oryzae (0.1%–0.8% solution) were used. The enzymatic treatment was carried out at 37 °C for 1–2 h. Carbogen was passed constantly through the solution. Further, the enzymes were removed, and enzymes residues were inactivated.

The isolated neurons were obtained under the microscopic control by repeatedly passing of the obtained brain slices in the solution with adding of 1.25 mmol/L CaCl₂ and 0.55 mmol/L MgCl₂ through the glass micropipette with a pore diameter of about 100 µm. The obtained suspension of neurons was added to the MEM, supplemented with 5% bovine serum (other types of sera were used as well). In this environment, the cells remained for 3–4 h without noticeable changes in their morphological characteristics. Some of these slices were left in the solution for enzymatic removing by “washing” with a constant passage of the carbogen. These slices could be used to obtain isolated neurons for 6–8 h. The described sequence of procedures enabled obtaining neurons of the characteristic pyramidal shape: elongated soma with preserved apical and basal dendrites of the second and third orders. The diameters of such cells were about 15–20 µm, and the lengths were about 20–40 µm (Fig. 1A). The neurons obtained using such procedures survived in culture for 42–45 days. Cultured neurons were considered as controls. Standard package MATLAB was used for mathematical processing of the obtained experimental data. Under the influence of the antagonists were studied such effects:
degrees of currents suppression and blockers removal by “washing”, “dose–effect” dependencies, the antagonists’ influences on activated and inactivated receptor; the kinetics of the antagonists’ action and removal, as well as the analysis of dissociation constants (for blockers with reversible action) and Hill plot. The details of the described methods are supported by the patents [19, 22, 23].

RESULTS AND DISCUSSION

The influence of toxins – the derivatives of phenols and indoles from *A. lobata* – on chemoactivated transmembrane electric currents in brain neurons. In the described experiments, chemo-activated transmembrane electric currents were studied in the membranes of rat hippocampal neurons, as well as the influence on them by AR-V and its toxins – the derivatives of phenols and indoles coupled with different polyamine substituents: AR, ARN-1, ARN-2. The purpose of conducting these experiments was to investigate the electrophysiological characteristics, the mechanisms of gCRC functioning, and to study the details of Glu-R chemical structure.

The hippocampal neurons under study (about 260 cells) demonstrated electrical excitability. The depolarizing shift of the membrane potential from -100 mV to -30 mV initiated transmembrane ion currents: the initial inward TTX-sensitive sodium currents and outward potassium currents. It was also possible to activate input ion currents in the membranes at holding potentials from -100 mV to +20 mV by the application of agonists (A), such as L-glutamate (GLU) and KK (Fig. 2). The kinetics of currents activated by these agonists was different. After the application of GLU and QL, the wave-like electrical currents were registered. Following rapid activation of currents to the maximum (about 10 ms), their decline caused by desensitization of receptors occurred. After

![Fig. 2. The results of the influence of *A. lobata* antagonists on kainate-activated currents: A – kainate-activated currents blocking with 10^-4 g/mL integral venom AR-V; B – kainate-activated currents blocking with argiopin 1.6*10^-2 mol/L. Concentration: kainate (KK) 1 mmol/L. V_{hold} = -100 mV. Records A and B were made on two different neurons](image-url)
the application of KK initiated inward currents, their amplitudes increased to the stationary levels, and such currents were non-desensitized [1]. At the background of such stationary non-desensitized currents, it was convenient to register the effects of different receptor antagonists [1, 18]. At the same time, all these agonists (GLU, KK, QL) activate the same membrane receptor system [18]. These effects allowed investigating the blocking characteristics of glutamate receptors antagonists in rat hippocampal pyramidal neurons using KK as an agonist of these receptors. Inward ionic currents were also activated under the influence of other agonists: villardiin, domoate, homocysteate. In addition, both types of neurons (freshly dissociated and taken from culture condition) were sensitive to inhibitory mediators – glycine and γ-butyric acid (GABA). None of tested Glu-R blockers (AR-V, AR, ARN-1, ARN-2) initiated chemo-activated currents by themselves. They did not affect the characteristics of electrically excitable currents (sodium input and potassium output currents) [1, 19].

**GLU- and KK-activated currents blocked by AR-V, AR and other similar antagonists.** In our previous experiments all studied substances – AR-V, AR, ARN-1, ARN-2 – demonstrated similar properties [20]. The amplitudes of transmembrane ionic currents activated by GLU, KK, QL decreased (sometimes to zero) after the application of AR blockers to the rat hippocampal membrane under the voltage-clamp conditions. The kinetics of activation and desensitization (in the case of GLU, QL) of these currents were not affected by these antagonists (Tables 1, 2).

**Blocking properties of integral venom** *A. lobata* **and argiopin.** In a series of experiments, the properties of some glutamate receptor blockers from *A. lobata* spiders were investigated. The amplitudes of GLU-, KK-, QL-activated ion currents were decreased after the application of integral venom (AR-V) and its main acting component – AR to the neuronal membrane. The action of AR-V and AR on stationary KK-activated ion currents is demonstrated in Fig. 2. The amplitudes of stationary KK-activated currents decreased exponentially under the action of AR-V and AR, revealing the kinetics of action of *A. lobata* antagonists.

The antagonists were used in the following concentrations: AR-V $10^{-4}$ g/mL, AR within the concentrations $5 \times 10^{-8}$–$1 \times 10^{-2}$ mol/L. At these concentrations, AR-V and AR never suppressed GLU, KK, QL-activated currents completely. Averaged amplitude of non-blocking AR-V components was 14.4% of the original value for KK-activated currents. AR reduced the amplitudes of KK-activated currents to 19% of the control values.

The blocking action of AR-V and AR on GLU-, KK-, QL-activated currents was reversible. After blocking of KK-activated currents by AR-V and AR, these substances could be removed by “washing” with Ringer’s solution with (partial) restoring of the response amplitudes. The amplitudes of the recovered chemo-activated currents were always lower than the control ones. For KK-activated currents, the averaged recovered amplitude was only 34% after the AR-V “washing”, but under AR action, it was significantly higher and reached 77% of the control value after “washing” (Fig. 2, Table 1). The amplitudes of chemo-activated currents were effectively recovered by “washing” with Ringer’s solution or solutions containing agonists (GLU, KK). The recovery characteristics did not depend on whether or not there was an agonist in the Ringer’s solution.

Responses recovered after the blocker action can be blocked and repeatedly washed with AR-V and AR (Fig. 3A, B). This procedure can be repeated several times until the cell death. With each such AR-V or AR application the degree of “washing” decreased. Thus, the effects of AR-V and AR were partially reversible.
Table 1. General characteristics of gCRC antagonists influences on kainate-activated ionic currents (integral venom from A. lobata and toxins isolated from it)

| Antagonist | Effect | Blocking: irreversible | Currents' amplitudes suppression (%) | Currents' amplitudes recovery (%) | Potential dependence of Ant influence on gCRC | Antagonists influences on gCRC | Antagonists influences on kinetics | Kinetics of antagonist influence: described by 1 or 2 exponents |
|------------|--------|------------------------|--------------------------------------|----------------------------------|---------------------------------------------|-------------------------------|----------------------------------|--------------------------------------------------|
| AR-V       | +      | 14.4                   | 34.0                                 | +                                | +                                           | +                             | -                                | 1 2                                              |
| AR         | +      | 19.0                   | 77.0                                 | +                                | +                                           | +                             | -                                | 1 2                                              |
| ARN-1      | +      | 44.0                   | 56.0                                 | +                                | +                                           | +                             | -                                | 1 2                                              |
| ARN-2      | +      | 22.0                   | 47.0                                 | +                                | +                                           | +                             | -                                | 1 2                                              |

Table 2. Kinetic characteristics of kainate-activated ionic currents blocking by integral venom and toxins from A. lobata

| Antagonist | Constant rates for velocity of blocking (direct reaction) | Velocity of recovery of currents' amplitudes | Dissociation constant |
|------------|----------------------------------------------------------|----------------------------------------------|-----------------------|
| Argiopin (AR) | 1.6·10³ L/(mol·s) | 0.85·10⁴ L/(mol·s) | 4.2·10⁻² s⁻¹ | 2.5·10⁻⁶ mol/L |
| Argiopinin 1 (ARN-1) | 3.3·10³ L/(mol·s) | 1.6·10⁴ L/(mol·s) | 7.9·10⁻² s⁻¹ | - |
| Argiopinin 2 (ARN-2) | 2.9·10³ L/(mol·s) | 0.52·10⁴ L/(mol·s) | 3.1·10⁻² s⁻¹ | - |

Partial reversibility of AR action made it possible to obtain dose–effect characteristics of KK-activated currents before the influence of antagonists and after the action of small amounts of AR, and then to compare them (Fig. 4A). In both cases such dose–effect characteristics were isotherms of “single binding”. The $K_d$ value of control dependence was 5.0·10⁻⁴ mol/L. Under the action of 10⁻⁵ mol/L AR, the maximal amplitudes of the KK-activated currents decreased 2.7 times; the character of dependence did not change, and $K_d$ of KK interaction with membrane receptors slightly decreased: $K_d = 2.4·10⁻⁴$ mol/L. Thus, AR did not compete with KK for receptor binding sites. The results of similar experiments indicated that there is no competition for receptor binding sites between GLU and AR.

Since AR blocking of the GLU- and KK-activated currents was incomplete, we investigated current–voltage characteristics (I–V characteristics) of those components of currents that were not blocked by AR. Characteristics I–V of these components of the
GLU- and KK-activated currents remained linear, as before toxin influence. The reversal potentials of currents did not change either, for example, for KK-activated currents they were about +30 mV.

**Fig. 3.** Peculiarities of the action of integral venom from *A. lobata* (AR-V): A, B – open channel blocking and reversibility of action (explanation see in text). Concentration: kainate (KK) 1 mmol/L, AR-V – 10^-4 g/mL. V\_hold = -100 mV; C – argiopin AR causes blocking of open state of kainate-activated ion channels. After receiving the control response, the neuron was kept in AR for 3 min, and then kainate was added against AR background. Concentration: kainate (KK) 1 mmol/L, AR – 1.6 \times 10^{-2} mol/L. V\_hold = -100 mV. Time of “washing” 15 s.

**Kinetics of argiopin influence on KK-activated currents and calculated values of blockage characteristics.** We have calculated kinetic parameters of blocking of stationary KK-activated currents by AR (Fig. 4B). The kinetics of AR action was also
detected at the background of stationary KK-activated currents. The formal description of blocking process of the receptor and AR “washing” revealed that the stationary current blocking process was satisfactorily described only by the sum of two exponential components. The experimental curves were logarithmized and the parameters of a slower exponent were found by the least square method. The time constants $\tau_1$ and $\tau_2$ of both exponents decreased with the increase of AR concentration. By plotting the dependence of $1/\tau$ on the concentration, we estimated the values of direct constants' rates ($k_1, k_2$) for the interaction of AR with non-NMDA receptors. The values $k_1$ and $k_2$ for AR were equal to: $k_1 = 1.6 \times 10^3$ L/(mol·s), $k_2 = 0.85 \times 10^4$ L/(mol·s). The kinetics of currents' amplitudes restoring during the “washing” of AR were one-exponential. Time constant rate for currents' recovery $\tau_\_was not dependent on the toxin concentration. The constant rate for currents' recovery for partial AR "washing" was $1/\tau_\_ = 4.2 \times 10^{-2}$ s$^{-1}$ (Table 2).

From the ratio of inverse and direct rate constants of AR interaction with receptors it was possible to calculate the value of dissociation constant for this toxin, which was $K_\D = 2.5 \times 10^{-6}$ mol/L. This value coincided with $K_\D$ value obtained in another way. Partial reversibility of AR action allowed obtaining the dose–effect of the action of AR — the dependence for suppression degree of KK-activated currents by this toxin. This dependence was an isotherm of single binding with $K_\D = 5.04 \times 10^{-6}$ mol/L. However, since AR effect was not completely reversed, the second estimation seems to be less reliable and therefore, the first value for further calculations was used.

Data analysis of the dose–effect presented on the Hill plot is usually used for the quantitative description of the process of toxin interaction with a receptor (Fig. 4C). According to representation of these data, the abscissa axis defines the values of lg C, where C is the concentration of the toxin, and the y-axis means lgθ/1-θ, where θ is the expression $\theta = \frac{l(o) - l(c)}{l(o)}$, in which $l(o)$ is the amplitude of the chemo-activated current in the absence of the toxin, and $l(c)$ is the current amplitude in the solution containing the toxin in C concentration. The calculated data formed a direct line; from the tangent of it, the Hill coefficient $n$ could be calculated. The Hill coefficient was $n = 0.86$ for blocking of KK-activated currents by AR. This value corresponds to the binding of one AR ligand to one receptor molecule, and indicates the absence of cooperativity of this process. From this plot, it was possible to determine $K_\D$ of AR interaction with the receptor at the point of plot intersection with the abscissa. As can be seen, these values approximately coincided with the values given above.

The influence of AR-V and argiopin on the activated glutamate channel-receptor complex and the dependence of these effects on the potential. The results of our experiments evidenced that both AR-V and AR blocked the gCRC only in the activated state (Figs. 3A, B). In these experiments, the agonist (KK) was “washed” with Ringer's solution after receiving the control response; then the cell was kept in blocker solutions for 2–3 min. After that, at the background of the blocker, we applied the agonist and recorded the resulting current. As one can see in Figs. 3A, C, this response was characterized by a peak in the initial phase that was followed by current decrease to a new steady state level. The current peak indicated that at least most of the gCRCs were not blocked after antagonists' influence. The process of further blocking of activated gCRCs reflected the phase of currents' decline. According to their kinetics, the processes in Fig. 3A and Fig. 3C do not differ significantly.
The action of AR-V and AR on chemo-activated currents depended on holding potential in the same way. The processes of currents blocking by the studied antagonists became slower with membrane depolarization. Furthermore, the effect was more pronounced with higher level of membrane depolarization. Blocking process of KK-activated ion currents by AR-V and AR at holding potential -30 mV is shown in Fig. 5A.
Fig. 5. Reduction of blocking effect of *A. lobata* integral venom (AR-V) (*A*) and argiopin (AR) (*B*) with reducing the level of holding potential. Concentrations: kainate (KK) 1 mmol/L, AR-V – 10^{-4} g/mL, AR – 1.6 \times 10^{-2} \text{mol/L}. Time of “washing” in Ringer’s solution was 30 s (*A*) and 15 s (*B*). Records *A* and *B* were made on two different neurons; *C* – increase of amplitudes of currents’ components that were not blocked by AR during membrane depolarization; *D* – increase of time constants of argiopin AR blocking effect with holding potential reducing; 1 and 2 were the slow and fast components, respectively.

Рис. 5. Зменшення блокуючої дії цілісної отрути з *A. lobata* (AR-V) (*А*) і аргіопіну (AR) (*В*) у разі зниження рівня підтримуваного потенціалу. Концентрації: каїнату (KK) 1 ммоль/л, AR-V – 10^{-4} г/мл, AR – 1.6 \times 10^{-2} \text{моль/л}. Тривалість відмивання розчином Рінґера становила 30 с (*А*) і 15 с (*В*). За-писи *А* і *В* зроблені на двох різних нейронах; *С* – збільшення амплітуди компоненти струму, що не блокувався AR під час деполяризації мембрани; *D* – збільшення постійних часу блокування аргіопіном у разі зниження підтримуваного потенціалу; 1 і 2 – відповідно повільний і швидкий компоненти

can be seen, with holding potential of -30 mV the blocking process not only significantly slowed down, but its degree became much smaller. The amplitudes of the KK-activated currents after the blocking demonstrated virtually no difference with the amplitudes in
control. In case of AR-V and AR “washing” with Ringer’s solution, the amplitudes of KK-activated currents were recovered almost completely. A decrease in current amplitudes was very slight in case of $V_{\text{hold}} = -30$ mV. It was so slight that in some cases virtual absences of blocking effects were registered. A decrease of the blockers’ efficiency with the membrane depolarization is illustrated in Fig. 5C, D. As one can see in the plots, the amplitudes of non-blocked by AR-V or AR components of KK-currents increased with the membrane depolarization. Fig. 5D illustrates the fact that both time constants of AR blocking, both fast and slow, increased with membrane depolarizing.

**Other antagonists of glutamate receptors’ from A. lobata venom.** Toxins from A. lobata venom were obtained using HPLC [12, 13]. The isolated fraction 6 was studied. After the application of fraction 6 to the membrane, it was found that this fraction demonstrated highly expressed irreversible effect blocking both KK- and GLU-activated currents. In Prof. Grishin’s research group, the content of fraction 6 was purified and studied, eventually toxins called arginopinin-1 (ARN-1) and argiopinin-2 (ARN-2) were obtained. Their chemical structures were further deciphered [12, 13, 20].

The substances ARN-1 and ARN-2 demonstrated similarity in their action (Fig. 6A, B, C, D). Both of them caused the decline of GLU- and KK-activated ion currents amplitudes acting in small quantities of $10^{-5}$–$10^{-6}$ mol/L. Differences between them were in the quantitative characteristics of blocking action. Thus, the maximal levels of blocking by these toxins of chemo-activated currents were different. ARN-1 reduced KK-activated currents to 44% of the control value, ARN-2 reduced them to 22%. In experiments with “washing” of these toxins with Ringer’s solution, the amplitudes of KK-activated ion currents were recovered unequally: after ARN-1 influence – up to 56%, after ARN-2 influence – up to 47% of the initial value. Thus, after ARN-2 “washing”, the amplitude of KK-activated currents was recovered more than twice (Fig. 6C, D), whereas after ARN-1 “washing”, KK-activated currents were hardly restored at all (Fig. 6A, B). The above results indicate that the irreversibility of fraction 6 action is mainly due to the presence of ARN-1.

Similar to AR, blocking properties of ARN-1 and ARN-2 depended on the holding potential. By reducing the holding potential level to -30 mV, the rate of KK-activated currents blocking by these toxins slowed down, and the degree of blocking decreased. These toxins blocked the gCRC in the activated state, so, this property was similar to that of the AR.

We also studied the kinetics of ARN-1 and ARN-2 interaction with gCRC. The schemes of experiments were completely similar to those described above for AR-V and AR; the kinetics of these toxins action was investigated against the background of KK-activated currents. It was registered that the kinetics of blocking by ARN-1 and ARN-2 were similar to AR: the processes of currents blocking were described satisfactorily by the sum of two exponents; the process of “washing” of toxins – by one exponent. Likewise for AR, the kinetic parameters of the toxins’ interactions with gCRC were calculated (see above). Binding constants of toxins with gCRC were equal to, respectively:

\[ k_1 = 3.3 \cdot 10^3 \text{ L/(mol-s)}, \quad k_2 = 1.6 \cdot 10^4 \text{ L/(mol-s)} \text{ for ARN-1, and} \]
\[ k_1 = 2.9 \cdot 10^3 \text{ L/(mol-s)}, \quad k_2 = 0.52 \cdot 10^4 \text{ L/(mol-s)} \text{ for ARN-2} \]

The velocity of “washing” was characterized by the value $1/\tau_-$. It was inverted to the time constant rate of toxin “washing” $\tau_-$. The values of kinetic parameters of KK-activated currents blocking by different toxins are shown in Table 2.
Fig. 6. Influence of argiopinins – argiopinin 1 (ARN-1), argiopinin 2 (ARN-2) on kainate-activated currents: 

A, B – irreversible blocking of kainate-activated currents by argiopinin 1 (ARN-1) (explanations in text); C, D – blocking of KK-activated currents by argiopinin 2 (ARN-2). Slight restoration of currents’ amplitudes was registered during ARN-2 removal by “washing” (explanations in text)

Рис. 6. Результати впливу аргіопінінів – аргіопініну 1 (ARN-1), аргіопініну 2 (ARN-2) на каїнатактивовані струми: 

A, B – необоротне блокування каїнатактивованих струмів аргіопініном 1 (ARN-1) (пояснення в тексті); C, D – блокування каїнатактивованих струмів аргіопініном 2 (ARN-2). У разі видалення ARN-2 способом “відмивання” зареєстровано слабке відновлення амплітуд струмів (пояснення в тексті)
CONCLUSIONS

Physiological effects of AR-V, AR, ARN-1, ARN-2 reflect the biological necessities of A. lobata. These spiders need to paralyze their victims, but they do not kill them. That is why some of toxins demonstrated reversible effects in the performed experiments. The degree of reversibility of AR’s action is the highest among all studied toxins: AR suppressed KK-activated currents to 19.0% of control value, and it can be “washed” to 77% of this value (see Table 1). Other venom components contribute to physiological reaction of victim as well, but because of their minor quantities, they only modulate the effects of the main active component.

The results of action of antagonists from spiders A. lobata (integral venom AR-V and three toxins – AR, ARN-1, ARN-2) on gCRC in rat hippocampal membranes are presented. In the studied concentrations under the voltage-clamp conditions these antagonists decreased the amplitudes of GLU, KK, QL-activated currents. The kinetics of activation and desensitization (in case of GLU, QL) of currents were not affected by the antagonists. AR-V, AR, ARN-1 and ARN-2 never suppressed GLU, KK, QL-activated currents completely (Table 1). The differences between them were in the quantitative characteristics of their blocking action, kinetics of blocking and removing of the antagonists by “washing” in Ringer solution. Under the antagonists’ influence the following effects were studied: degrees of currents suppression and their removal by “washing”, “dose–effect” dependencies, the antagonists’ influences on an activated and inactivated receptor, kinetics of antagonists’ action and removal (Tables 1, 2). AR demonstrated a reversible effect with $K_d$ value equal to $2.5 \times 10^{-6}$ mol/L. The calculated value of Hill coefficient for AR was 0.86, which means that one AR molecule interacts with one gCRC molecule without the cooperativity.

The action of all studied substances depended on holding transmembrane potential. Such dependence of blocking properties on potential suggests that it was the ion channel of gCRC that was blocked. All studied toxins from A. lobata – AR, ARN-1, and ARN-2 blocked chemo-activated currents by binding to the glutamate receptor of gCRC in the activated state. The regularity in kinetics of gCRC antagonists’ actions was revealed. When the concentration of an antagonist in a solution was increased, the velocity of blocking increased too. Thus, the rate of blocking increased with the increasing of toxins’ concentrations. The obtained results enable us to make conclusions about the mechanisms of these antagonists’ influence on gCRC and compare the caused effects. More details about the mechanisms of these toxins’ activities were presented in [20]. The registered regularities in their effects that could be used as a basis for novel types of qualitative and quantitative analysis were shown [21]. The developed methods that enabled obtaining the results important for the applications in research and technology are protected by the patents of Ukraine [19–23].

ACKNOWLEDGEMENTS

The author is grateful to people with whom this long-lasting research work became possible: father – aviation engineer, Assoc. Prof., PhD. (National Aviation University of Ukraine), mother – Prof., Dr. Sci. in entomology Z.F. Klyuchko (I.I. Schmalhausen Institute of Zoology, National Academy of Sciences of Ukraine), and great teachers in neurophysiology – Prof. O.A. Krishtal (O.O. Bohomolets Institute of Physiology, National Academy of Sciences of Ukraine) and Prof. N. Akaike (Tokyo University, Japan), as well as to the colleagues A.Ya. Tsyndrenko and N.I. Kiskin.
COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Human Rights: This article does not contain any studies with human subjects performed by the any of the authors.

Animal studies: All institutional, national and institutional guidelines for the care and use of laboratory animals were followed.

1. Akaike N., Kawai N., Kiskin N.I., Kljuchko E.M., Krishtal O.A., Tsyndrenko A.Ya. Spider toxin blocks excitatory amino acid responses in isolated hippocampal pyramidal neurons. Neurosci. Lett., 1987; 79: 326–330. [DOI: https://doi.org/10.1016/0304-3940(87)90453-8; Google Scholar]

2. Aramaki Y., Yashuhara T., Higashijima T., Yoshioka M., Miwa A., Kawai N., Nakajima T. Chemical characterization of spider toxins JSTX and NSTX. Proc. Japan Academy, 1986; 62(9): 359–362. [DOI: https://doi.org/10.2183/pjab.62.359; Google Scholar]

3. Bateman A., Boden P., Dell A., Duce I.R., Quicke D.L., Usherwood P.N.R. Postsynaptic block of a glutaminergic synapse by low molecular weight fraction of spider venom. Brain Res., 1985; 339(2): 237–244. [DOI: https://doi.org/10.1016/0006-8993(85)90088-5; Google Scholar]

4. Biner O., Trachsel C., Moser A., Köpp L., Langenegger N., Kämpfer U., von Ballmoos C., Nentwig W., Schürch S., Schaller J., Kuhn-Nentwig L. Isolation, N-glycosylations and Function of a Hyaluronidase-Like Enzyme from the Venom of the Spider Cupiennius salei. PLoS One, 2015; 10(12): e0143963. [DOI: https://doi.org/10.1371/journal.pone.0143963; PMid: 26630650; Google Scholar]

5. Budd T., Clinton P., Dell A., Duce I.R., Johnson S.J., Quicke D.L., Usherwood P.N.R., Usoh G. Isolation and characterisation of glutamate receptor antagonists from venoms of orb-web spiders. Brain Res., 1988; 448(2): 30–39. [DOI: https://doi.org/10.1016/0006-8993(88)91098-0; Google Scholar]

6. Casewell N.R., Wüster W., Vonk F.J., Harrison R.A., Fry B.G. Complex cocktails: the evolutionary novelty of venoms. Trends Ecol Evol, 2013; 28(4): 219–29. [DOI: https://doi.org/10.1016/j.tree.2012.10.020; PMid: 23219381; Google Scholar]

7. Cavigliasso F., Mathé-Hubert H., Kremmer L., Rebuf C., Gatti J.L., Malasa T., Colinet D., Poirié M. Rapid and Differential Evolution of the Venom Composition of a Parasitoid Wasp Depending on the Host Strain. Toxins (Basel), 2019; 11(11): 629. [DOI: https://doi.org/10.3390/toxins11110629; PMid: 31671900; Google Scholar]

8. Cordell G.A., Brossi A. (Eds.). Chemistry and Pharmacology. The Alkaloids. USA: Academic Press, 1994. 280 p.

9. Daly N.L., Wilson D. Structural diversity of arthropod venom toxins. Toxicon, 2018; 152: 46–56. [DOI: https://doi.org/10.1016/j.toxicon.2018.07.018; PMid: 30040988; Google Scholar]

10. Fortschrritte der Chemie organischer Naturstoffe. In: Progress in the Chemistry of Organic Natural Products. (Ed.) W. Herz, G. W. Kirby, R. E. Moore, W. Steglich, Ch. Tamm. USA: Springer Science & Business Media, 2012; 66: 332 p.

11. Grishin E. Spider toxins active on purinergic P2X3 receptor. Toxicon, 2016; 116: 72. [DOI: https://doi.org/10.1016/j.toxicon.2016.01.003; Google Scholar]

12. Grishin E.V., Volkova T.M., Arseniev A.S. Antagonists of glutamate receptors from the venom of Argiope lobata spider. Bioorganicheskaya chimia, 1988; 14(7): 883–892. (In Russian)

13. Grishin E.V., Volkova T.M., Arsenyev A.S., Reshetova O.S., Onoprienko V.V., Magazanik L.G., Antonov S.M., Fedorova I.M. Structural and functional characteristics of argiopin – ion channel blocker from venom of spider Argiope lobata. Bioorganicheskaya chimia, 1986; 12(8): 1121–1124. (In Russian)
14. Hashimoto Y., Endo Y., Shudo K., Aramaki Y., Kawai N., Nakajima T. Synthesis of spider toxin JSTX-3 and its analogs. *Tetrah. Lett.*, 1987; 28(30): 3511–3514. [DOI: https://doi.org/10.1016/S0040-4039(00)96340-8; Google Scholar]

15. Herzig V. Arthropod assassins: Crawling biochemists with diverse toxin pharmacopeias. *Toxicon*, 2019; 158: 33–37. [DOI: https://doi.org/10.1016/j.toxicon.2018.11.312; PMid: 30496730; Google Scholar]

16. Jackson H., Usherwood F.N.R. Spider toxins as tools for dissecting elements of excitatory amino acids transmission. *Trends in Neurosci.*, 1988; 11(6): 278–283. [DOI: https://doi.org/10.1016/0166-2236(88)90112-9; Google Scholar]

17. Kachel H.S., Buckingham S.D., Sattelle D.B. Insect toxins – selective pharmacological tools and drug/chemical leads. *Curr Opin Insect Sci.*, 2018; 30: 93–98. [DOI: https://doi.org/10.1016/j.cois.2018.10.001; PMid: 30553492; Google Scholar]

18. Kissin N.I., Krishtal J.A., Tsyndrenko A.Ya. Excitatory amino acid receptors in hippocampal neurons: kainate fails to desensitize them. *Neurosci Lett.*, 1986; 63(2): 225–230. [DOI: https://doi.org/10.1016/0304-3940(86)90360-5]

19. Klyuchko O. M. Method of cells’ dissociation. – Patent UA 130672 U, G01N 33/00, C12Q 1/02. Priority: 22.03.2018, 2019; 158: 33–37. [DOI: https://doi.org/10.1016/j.toxicon.2018.11.312; PMid: 30496730; Google Scholar]

20. Klyuchko O.M. Comparative analysis of Araneidae venoms and toxins: chemical structures and electrophysiological effects. *Biol. Stud.*, 2020: 14(1); 89–104. [DOI: https://doi.org/10.30970/sbi.1401.606; Google Scholar]

21. Klyuchko O.M., Biletsky A.Ya. Computer recognition of chemical substances based on their electrophysiological characteristics. *Biotechnologia Acta*, 2019; 12(5): 5–28. [DOI: https://doi.org/10.15407/biotech12.05.005; Google Scholar]

22. Klyuchko O.M., Tsyndrenko A.Ya. Method of dissociation of hippocampal cells. Patent USSR 1370136, С12N 5/00. Priority: 31.01.1986; Issued: 30.01.1988, Klyuchko O.M., Biletsky A.Ya., Nakajima T.; PMid: 30529476; Google Scholar

23. Hashimoto Y., Endo Y., Shudo K., Aramaki Y., Kawai N., Nakajima T. Synthesis of spider toxin JSTX-3 and its analogs. *Tetrah. Lett.*, 1987; 28(30): 3511–3514. [DOI: https://doi.org/10.1016/S0040-4039(00)96340-8; Google Scholar]

24. Klyuchko O.M., Biletsky A.Ya., Navrotskyi D.O. Method of bio-sensor test system application. – Patent UA 129923 U, G01N33/00, G01N33/50, C12Q 1/02. Priority: 22.03.2018, 2019; 158: 33–37. [DOI: https://doi.org/10.1016/j.toxicon.2018.11.312; PMid: 30496730; Google Scholar]

25. Kusano T., Suzuki H. Polyamines: A Universal Molecular Nexus for Growth, Survival, and Specialized Metabolism. USA: Springer, 2015. 336 p. [DOI: https://doi.org/10.1007/978-4-311-55212-3; Google Scholar]

26. Lajoie D.M., Zobel-Thropp B.A., Delahaye J., Roberts S., Kumirov V.K., Bandarian V., Binford G.J., Cordes M.H.J. The chemistry and functional diversity of spider phospholipase D toxins. *Toxicon*, 2016; 116: 79. [DOI: https://doi.org/10.1016/j.toxicon.2016.01.025; Google Scholar]

27. Lee S.Y., Kim S.T., Jung J.K., Lee J.H. A comparison of spider communities in Bt and non-Bt rice fields. *Environ Entomol.*, 2014; 43(3): 819–827. [DOI: https://doi.org/10.1603/EN12259; PMid: 24874159; Google Scholar]

28. Rádis-Baptista G., Konno K. Arthropod Venom Components and Their Potential Usage. *Toxins* (Basel), 2020; 12(2): 82. [DOI: https://doi.org/10.3390/toxins12020082; PMid: 31991714; Google Scholar]

29. Senji Laxme R.R., Suranse V., Sunagar K. Arthropod venoms: biochemistry, ecology and evolution. *Toxicon*, 2019; 158: 84–103. [DOI: https://doi.org/10.1016/j.toxicon.2018.11.433; PMid: 30529476; Google Scholar]

30. Schwartz E.F., Mourão C.B., Moreira K.G., Camargos T.S., Mortari M.R. Arthropod venoms: a vast arsenal of insecticidal neuropeptides. *Biopolymers*, 2012; 98(4): 385–405. [DOI: https://doi.org/10.1002/bip.22100; PMid: 23193602; Google Scholar]

31. Walker A.A., Robinson S.D., Yeates D.K., Jin J., Baumann K., Dobson J., Fry B.G., King G.F. Entomo-venomics: the evolution, biology and biochemistry of insect venoms. *Toxicon*, 2018; 154: 15–27. [DOI: https://doi.org/10.1016/j.toxicon.2018.09.004; PMid: 30267720; Google Scholar]
ОТРУТА Й ТОКСИНИ З ARGIOPE LOBATA: ЕЛЕКТРОФІЗІОЛОГІЧНЕ ДОСЛІДЖЕННЯ

О. М. Ключко

Національний авіаційний університет, просп. Любомира Гузара, 1, Київ 03058, Україна
e-mail: kelenaxx@nau.edu.ua

Мета цієї роботи – представити отримані експериментальні дані про дію токсинів та отрути павуків Argoipe lobata на глутаматний канало-рецепторний комплекс. Каїнат було застосовано як агоніст глутаматного канало-рецепторного комплексу, оскільки він ініціював у мембранах гіпокампа щурів трансмембрани вхідні іонні електричні струми, що не десенситизувалися. На тлі таких струмів зручно досліджувати дію антагоністів. Дослідники застосували відомі іонні струми та антагоністи глутаматного канало-рецепторного комплексу з A. lobata. Проведено електрофізіологічні експерименти, під час яких усі хімічні речовини вплинули на мембрану перфузованіх пірамідних нейронів гіпокампа, застосовуючи методику "фіксації концентрацій". Для одноелектродної реєстрації за умов фіксації потенціалу застосовували стандартну електронну схему. Усі досліджені речовини – цілісна отрута, аргіопін, аргіопінін 1, аргіопінін 2 – мали подібні властивості. Амплітуда іонних струмів, активованих глутаматом, каїнатом, квісквалатом зменшувалася після впливу антагоністів на мембрану гіпокампа щурів за умов фіксації потенціалу. На кінетику активування та десенситизації (у випадку глутамату і квісквалату) струмів ці антагоністи не вплинули. Ефект аргіопіну досліджували в межах концентрацій 10^{-8}–10^{-2} моль/л, а ефект цілісної отрути – у концентрації 10^{-4} г/мл. Цілісна отрута й аргіопін у таких концентраціях вплинула на десенситизацію струмів. Середня амплітуда незаблокованих компонентів цілісної отрути становила 14,4 % від початкового значення каїнатактивованих струмів. Аргіопін зменшував амплітуду каїнатактивованих струмів до 19 % від контрольних значень прийнятих за 100 %. Ефекти речовин аргіопінін 1 і аргіопінін 2 були дуже схожими. Вони зумовлювали зниження амплітуди глутамат-і каїнатактивованих іонних струмів, діючи в малих концентраціях 10^{-8}–10^{-6} моль/л. Відмінності між ними полягали в кількісних характеристиках їхньої блокуючої дії. Ефект антагоністів досліджували на залежності "доза-ефект", впливових антагоністів на активовані та інактивовані рецептори, кінетиці впливу й видалення антагоністів, константи дисоціації. Зроблено висновки щодо механізмів дії цих антагоністів на глутаматний канало-рецепторний комплекс, а також проведено порівняння спричинених ними ефектів.

Ключові слова: Araneidae, отрута, токсин, антагоніст глутаматних рецепторів, трансмембраний електричний струм

Received / Одержано 03 April 2020
Accepted / Прийнято 22 May 2020
Published / Опубліковано 22 June 2020

ISSN 1996-4536 (print) • ISSN 2311-0783 (on-line) • Біологічні Студії / Studia Biologica • 2020 • Том 14/№2 • С. 39–56