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ABSTRACT

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Abstracts

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(18 animals) groups was studied by the mechanographic method using a Myobath II multi-channel tissue bath system. The control group was fed with standard laboratory rat diet (protein:fat:carbohydrate ratio 24%:6%:44%) with a free access to food and water. The rats of the experimental group at the 12th week were administrated with a high fat and high carbohydrate diet (protein:fat:carbohydrate ratio 16%:21%:54%), drinking water was replaced with a 20% fructose solution. Contractions of airway smooth muscle segments were induced by 30 mM potassium chloride solution (KCl, 30 mM). The amplitude of contractile responses to KCl served as the control (100%). The pharmacological effects of carbacholin (0.1-100 μM), salbutamol (0.1-100 μM), forskolin (0.1-10 μM) were tested.

As a result of the airway smooth muscles contractile activity study in experimental animals, it was found that the action of the nonselective cholinergic receptors agonist carbacholin (0.1–100 μM) causes a dose-dependent contraction of bronchial segments in control and experimental rats. The contractile responses amplitude in airway segments with removed epithelium in animals of the experimental group was higher than in the control group in the concentration range of 1–100 μM ($p<0.05$). Probably, the removing of the epithelium leads to a decrease in the action of dilatation factors released during the activation of cholinergic receptors. It was shown that in obesity caused by a high-fat diet, carbacholine enhances the contraction of the smooth muscles of the airways due to the calcium mobilization from cytosolic reserves, which stimulates the phosphorylation of myosin light chains. Activation of beta2-adrenergic receptors with salbutamol (0.1–100 μM) against the background of precontraction of segments by carbacholin (1 μM) caused a dose-dependent relaxation of the airway smooth muscle segments in rats of the control and experimental groups. The dilatation reactions of the segments with the removed epithelium from experimental rats decreased in the concentration range of 1–100 μM ($p<0.05$) when compared to the control group. One of the mechanisms for relaxing effect reducing on the bronchodilator action is the desensitization of beta2-adrenergic receptors, which occurs in obesity, due to increased expression of type 4 phosphodiesterase, which destroys cAMP. At the same time, contractile activity modulation in bronchial smooth muscles also depends on the acting mediator. It is possible that the activation of cholinergic mechanisms cancels the cAMP-dependent relaxation in smooth muscles. Against the background of the action of the adenylate cyclase activator forskolin, a dose-dependent relaxation of segments with the removed epithelium from rats of the control and experimental groups ($p<0.05$) occurred, more pronounced in the group of rats with MS.

Thus, the data obtained indicate that in animals with MS induced by a high-fat and high-carbohydrate diet, there are functional changes in the wall of the airways associated with a change in cAMP-dependent intracellular regulation.

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S9.654. Membrane activity of three-finger cobra cytotoxins depends on the critical amino acid residues in the N-terminal and central loops

Dubovskii P.V.[1]*, Utkin Y.N.[1]
[1] *Institute of Bioorganic Chemistry named after Shemyakin M.M. and Ovchinnikov Yu. A.;*
* pvdubov@ya.ru

Cobra cytotoxins (CT) are the main component of their venom, responsible for necrosis of the affected tissues. It is believed that this is based on the membrane activity of CT. Therefore, it is not surprising that CT possess cytotoxic and antibacterial activities, like many membrane-active peptides [1].

The database of protein structures (https://www.uniprot.org) contains more than 80 members of the CT family. These polypeptides, 59-61 amino acid residue long, belong to the three-finger protein family. Their characteristic structural feature is the presence of three beta-structural hairpins held together by 4 disulfide bonds. CT interact with lipid membranes through the termini of beta-hairpins, or loops. These regions are the most variable. Which substitutions are most favorable for membrane activity of CT?

For the first time, a group of researchers from Taiwan tried to answer this question in 1994 [2]. They found that the presence of either proline (Pro30) or serine (Ser28) residue at the terminus of the central loop of CT significantly influences their membrane activity. Therefore, all CT were divided into 2 groups: P-type (with Pro30) and S-type (with Ser28). And P-toxins exhibit stronger membrane activity than S-toxins. However, relatively recently, we found that the antibacterial activity and cytotoxicity of a number of CT, as well as their capability to induce calcein leakage from phospholipid liposomes, depend on the presence of a single proline residue (Pro8) or a pair of prolines (Pro8–Pro9) at the extremity of the N-terminal loop [3]. At the same time, CT with two prolines are significantly inferior in activity to those with one proline. The total number of CT with two prolines is at least 20. It should be noted that the dependence of activity on P- and S-substitutions in the second loop also remains.

With this in mind, all CTs, according to their membrane activity, can be divided into 4 groups, depending on the presence of critical amino acid residues at the extremities of the N-terminal and central loops of the molecule. Group-1 consists of CT with Pro8-Pro9 and Ser28 residues. Group-2 is represented with CT possessing Pro8-Pro9 and Pro30. Group-3 includes CT with Pro8 and Ser28. Group-4 - with Pro8 and Pro30. At the same time, the membrane activity of CT increases in the following order: group-1 < group-2 < group-3 < group-4. In many cases it is the membrane activity of CT that determines their antibacterial activity and cytotoxicity [2]. Thus the proposed classification of CT will allow to predict and compare their antibacterial/cytotoxic properties.

Currently, we are validating the proposed classification by expanding the number of CT for which antibacterial/cytotoxic activities have been determined.

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S9.655. Membrane characteristics of premotor interneurons of the defensive reflex after formation of anxiety-like behavior in snail

Chumarina A.I.[1,2]*, Arslanov A.I.[1,2], Silantyeva D.I.[1,2]
[1] *Institute of Fundamental Medicine and Biology;*
[2] *Kazan Federal University;*
* chumarinaadilya@yandex.ru

Anxiety, depending on the situation, can be a natural defensive reaction of the body or a serious pathology. Anxiety is often viewed as an exaggerated reaction of the body to an external threat, occurring in the absence of a life-threatening stimulus. The anxiety in mammalian is deeply rooted in their evolutionary past and can be found in invertebrates. So, in mollusks, an anxiety-like behaviour is expressed as an

unpredictable change in defensive and motor activity. It was determined that during the formation of long-term sensitization, which is close reaction such as fear, there is an increase in the excitability of the main elements of the neural network: sensory and command neurons, as well as an increase in the amplitude of EPSP. (Hochner et al., 1986). At the same time, it seems interesting how change the characteristics of neurons involved in the performing of defensive and motor responses in mollusks during anxiety. The aim of present study was to investigate how the anxiety-like behaviour affected the membrane characteristics of premotor interneurons of defensive reflex in snails.

The experiments were carried out on the terrestrial mollusk Helix Pomatia. To develop an anxiety-like behavior, 4 electrical stimuli were applied to mollusk on the area of the head each day with an interval of 1.5 hours within 3 days. Then the animals had 3 days of rest and after the same series of electrical stimulations were repeated. Anxiety-like behaviour was determined by behavioral tests such as locomotion, ommatophore and pneumostom retraction time. The electrophysiological activity of premotor interneurons was recorded on an isolated preparation of the nervous system of snails. The animals of the control group were kept in identical conditions, as well as the animals of the group when an anxiety-like behaviour was formed. The following parameters of the membrane characteristics of premotor interneurons were studied: membrane potential (Vm), amplitude and duration of excitatory postsynaptic potentials (EPSP). The registration of electrophysiological characteristics in snails that did not undergo the formation of anxiety-like behaviour was a control.

The study of the membrane characteristics of premotor interneurons showed that the membrane potential of premotor interneurons of snails with anxiety-like behaviour significantly shifted towards depolarization: Vm of the interneurons of animals with anxiety-like behaviour was -52.95±1.7 mV (n=9), while the Vm of the interneurons of animals in the control group was 59.2±2 mV (n=6) (P<0,05).

An analysis of the EPSP activity of premotor interneurons in snails showed that the total amplitude of EPSP recorded from these neurons in anxiety snails (1,01±0,1 mV) was slightly reduced compared to the total amplitude of EPSP in the premotor neurons of animals in the control group (1,3±0,3 мВ). The frequency of EPSP appearance in premotor interneurons in the group of animals with anxiety-like behaviour did not differ from that in the interneurons of control animals.

Thus, obtained data showed that, the rest membrane potential of premotor interneurons of defensive reflex in snails with anxiety-like behaviour was more depolarized compare with rest membrane potential in premotor interneurons in control snails, while the parameters of EPSP which reflected the activation of synapses from sensory neurons do not change significantly. The tendency to decreasing EPSP amplitude after the formation of an anxiety-like behavior can be explained by the depolarization of the membrane potential against which a part of the EPSP signal is lost. The mechanisms of the depolarization shift of the resting membrane potential during the formation of an anxiety-like state requires further study and is the goal of our next work.

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S9.656. Membrane correlates of learning in molluscs: the role of serotonin, glutamate, and nitric oxide in the formation of conditioned defensive reflexes in the grape snail

Gainutdinov Kh.L.[1]*
[1] *Kazan Federal University;*
* kh_gainutdinov@mail.ru

Processes of learning and memory underlie behavior change, and memory is one of the main cognitive functions of the brain. The mechanism for storing and/or remembering the received information constitutes memory. The issues of memory consolidation, including the formation of conditioned reflexes, remain relevant. Although the question about the mechanisms of learning and memory arose a long time ago, it has not yet been fully studied. Neuromodulation can have a significant impact on the formation of long-term memory [1]. Examples of neuromodulators in the simple nervous system of mollusks are serotonin, nitric oxide, and glutamate. The literature demonstrate that serotonin (5-HT) is the main mediator that modulate defensive behavior in mollusks. 5-HT, applied to the surrounding solution, causes several cellular changes that lead to an increase in the defensive reflex. In addition to the well-known role of 5-HT as a mediator in synaptic transmission, it was shown that it can perform integrative functions when released into the extracellular environment [1]. These results served as the basis for the application of 5-HT washing solution as a reinforcing stimulus for the purpose of creating cellular analogs of learning. By applying 5-HT to the solution washing the central nervous system, it is also possible to reproduce the electrophysiological correlates of plasticity.

Nitric oxide (NO) is known as one of the most important signaling molecules regulating the physiological functions of the body and cell metabolism. Much attention is drawn to the study of the role of NO in the mechanisms of learning and memory. NO-synthesizing neurons have been found in the nervous system of invertebrates, including mollusks. In mollusks, as in mammals, NO plays the role of an intercellular messenger and a signaling molecule in various parts of the nervous system. We have shown that both the NO donor sodium nitroprusside and the NO-synthase blocker L-NAME have a direct effect on the electrical characteristics of the premotor interneurons of the terrestrial snail. It is known that an essential role in the regulation of brain activity, particularly in memory processes, is played by L-glutamate, the main excitatory neurotransmitter in both vertebrates and many invertebrates. On the one hand, we studied the effect of changes in the content of serotonin, nitric oxide and glutamate on the formation of conditioned defensive reflexes of aversion to food and changing the environment, as well as on the reconsolidation of memory of these reflexes. On the other hand, we have conducted studies of the membrane mechanisms of the formation of conditional defensive reflexes in a mollusk with a simple nervous system – the terrestrial snail. To do this, we analyzed changes in the excitability of the premotor interneurons of the defensive reflex LPa3 and RPa3: the values of the membrane potential (Vm) and the threshold of action potential generation (Vt).

It was found that the application of 5-HT and the precursor of its synthesis 5-hydroxytryptophan (5-HTP) into the washing solution caused a decrease in the membrane potential (Vm) of LPa3 and RPa3 neurons, in both intact and trained animals. At the same time, in trained and sensitized snails, unlike intact snails, this application caused an increase in threshold potential (Vt). The results show that the responses (sensitivity) of premotor interneurons to extracellularly applied 5-HT or 5-HTP change after associative learning and long-term sensitization. It has been demonstrated that the reconsolidation of this contextually dependent memory of the situational conditioned reflex (CR) during reminder and simultaneous inhibition of protein synthesis does not occur if serotonin transmission is disrupted in the nervous system. It is shown that the development of the CR to the situation is accompanied by a depolarization shift and a decrease in the Vt of LPa3 and RPa3 neurons. No further Vm changes were detected after the reminder (initiation of reconsolidation) both with the subsequent injection of a protein synthesis blocker or saline solution. The Vt of these neurons decreases after learning and remains unchanged after the initiation of reconsolidation.

It was found that blocking the NMDA receptor with the MK-801 blocker in terrestrial snails accelerates the process of aversive learning. It has been shown that the application of a NO sodium nitroprusside donor into a solution washing the preparation of intact snails causes an increasing hyperpolarization of the membrane of premotor interneurons at 5.5 mV by the 10th minute. The application of the L-NAME