УДК 615.919

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VARIABILITY AND PROPERTIES OF HOST DEFENSE PEPTIDES FROM THE SKIN SECRETIONS OF ANURANS

Cationic antimicrobial proteins are an important part of innate nonspecific immunity. It is the first defensive level, which is inherent in almost all living organisms. The main objective of such proteins is the destruction of dangerous microorganisms (fungi, bacteria, viruses, parasites etc.). The skin of amphibians is a rich source of these molecules, which are produced and stockpiled in skin glands, which are usually located on the dorsal side of the body. Basically, they are spread over the surface of the body or grouped in special morphological structures – parotids. Currently the host defensive proteins were found in members of all families of amphibians, that suggests a connection among them with evolutionary advantages. Moreover, amphibian antimicrobial proteins can be used in modern medicine. Amphibians can become a rich source of biologically active agents and usage of them is very beneficial for pharmaceutical industry. These substances appeared to have much more abilities than it was believed before. For example, they can be used in methods of blood folding or antiviral therapy. Taking this into account, it is very promising to study antimicrobial proteins in Ukraine (from 15 anuran species of 5 families (Pelobatidae, Hylidae, Bufonidae, Ranidae and Bombinatoridae). This article describes the chemical structure and properties of the antimicrobial proteins presently known from the studies and their presence in different families of Anura. The main aim of the work is to show the variability of these substances in anurans to create a background for further investigations of amphibians' antimicrobial proteins in Ukraine and studying of their pharmaceutical potential.

Key words: antimicrobial peptides, amphibians, protective peptides, skin glands, secretions.

Introduction. The production of antimicrobial peptides is part of the innate immune system and is widely distributed in nature. This system was first discovered in the insects' hemolymph where the synthesis of antimicrobial peptides such as defensins and cecropins is induced in response to microbial infection. Skin secretions of many amphibian species have a wide range of biologically active substances with various functions (amines, proteins, steroids, water- and fat-soluble alkaloids, peptides). Nowadays, compounds with antiviral, antimicrobial, antifungal properties were identified.

The main types of pharmacologically active proteins, that have been discovered so far, are: caeruleins, tachykinins, bradykinins, thyrotropin-releasing hormone, bombesin-like and opioid peptides. In addition to peptides associated with mammalian hormones and/or neurotransmitters, amphibian skin contains numerous peptides with hemolytic properties. The glands that produce the poison and surrounded by myocytes are innervated by sympathetic nerve fibers. Adrenergic stimulation of myocytes in response to stress causes compression of serous cells and releasing of their content by holocrine type of secretion. Antimicrobial proteins synthesized by ribosomes in average length of 10 to 50 amino acid residues are widely distributed in nature. In unicellular organisms, plants and animals, including humans they are the first line of defense against harmful microorganisms [1].

Despite significant variations in the length and structure of host defense proteins (HDP) and no sustainable motives responsible for their activity, there are certain features that are common to all proteins investigated at present. They are as follows: – most of these proteins are cationic due to the presence of many lysine residues in their structure;- in aqueous solutions they usually do not have a secondary structure but take an α – helical conformation among phospholipid vesicles or in membrane-like solution. For example, in a 50 % trifluoroethanol/water solution their charge varies from +2 to +6 at pH 7; at least 50 % amino acid of protein are hydrophobic [2].

The skin of amphibians plays an important role in their survival and helps them to adopt to different environmental conditions [3].

Some of the peptides were found only in certain groups of amphibians. Brevinin-1, esculentin-1 and -2 and temporin were found in the Ranidae family in Eurasia and North America; ranalexin, ranateurin-2 and palustrin – only in the North American ranids; brevinin-2, tigerinin, japonicin nigrocin and melittin-like peptides were found only in the Eurasian toads [4].

Nowadays, there were notdiscovered two species of amphibians that have the same set of antibiotic proteins and there were not found two proteins with identical sequences in different species of frogs. Ortholog proteins (coded by homologous sequences, for the division of which the act of speciation has led: if a gene existed in some species, which divided into two types by divergence, the copies of the gene in daughterly species called orthologs) and paralogs (homologous sequences, for the division of which a doubling of the gene has resulted, if within the same organism as a result of chromosomal mutation was doubling gene, the copies are called paralogs) are formed because of mutations and processes of speciation. It has been found in many species of amphibians and have different aminoacid sequences in different species and often exhibit a different biological activity. Thus, the skin of amphibians are potentially important as a rich source for the discovery of new protein antibiotics.

There is no one mechanism that causes cell death by antimicrobial peptides (AMP), because their performance is always based on nonspecific interactions with the bacterial membrane, without an impact on the specific receptors (Yeaman and Yount, 2003).

It should be noted that the production of AMPs by some species of amphibians is seasonal and is influenced by thyroid hormones [5]. It can also be terminated by environmental factors or pesticides [6].

Almost everywhere we faced the problem of contamination of amphibians by pathogenic chytrid fungus Batrachochytrium dendrobatidis Longcore, Pessier & D. K. Nichols 1999 which reduces their populations. Numerous studies have shown that cleaned cytolytic proteins from the skin of frogs inhibit the growth of many types of adult cells and zoospores B. dendrobatidis in vitro [7].

Another group of organisms responsible for the deaths of large numbers of amphibians is ranaviruses (Irodoviridae). It turned out that some of the AMBs isolated from secretions of skin glands can inactivate viruses. These include brevinin-1 (affects herpesvirus types 1 and 2); caerin 1.1, caerin 1.9 and maculatin 1.1 (completely inhibit HIV in T cells); esculentin and ranateurin-2R-2R dermaseptin-B1, temporin A (rapidly inactivates frog virus type 3, pathogenic iridovirus) [8–10].

Even though the study of amphibian proteins was mainly focused on their antimicrobial properties, modern studies

show that they are also able to act as a cytokine-mediated immunomodulators, chemoattractants, insulin-releasing factors and have anticancer activity [11, 12].

The purpose of this work is to collect and systematize information from the literature on the distribution and properties of various host defense peptides in organisms of different families of tailless amphibians, especially the families represented in the batrahofauna of Ukraine. This work is the beginning of a series of studies of antimicrobial and other properties of the poisons of anurans in Ukraine, in order to study their perspectives to be used in medicine and pharmacology.

Ways of poisons extraction from the tailless amphibians. There are many ways of getting the poison from anurans, including lethal and non-lethal release. When lethal ways are used, the skin of animals that previously were subjected to decapitation, is removed, dried and ground to powder consistency for further use. Another lethal variant is to place a frog in a flask with anhydrous ether, which stimulates the secretion of the skin poison that is washed from the surface of the animal with deionized water.

Modern non-lethal methods include the usage of an electric current. The animal is subjected to short-term effects of the weak current which causes synchronous release of toxic secretions from the glands. Further, in sterile conditions, the secret is washed from the surface of animal with deionized water [13].

Another non-lethal way is to stimulate poison release by chemical injection: the animal gets a hydrochloride injection into a lymphatic bag (40 nmol/1 g body weight) and is then placed in 100 ml of distilled water for 15 minutes. Poison secret is released into the aquatic environment. After the frog is removed trifluoroacetic acid (TFA) is added and then the solution is frozen for later use [14].

Distribution of skin antimicrobial peptides in the anuran families. It is a common misconception that all frogs synthesize and release cytotoxic peptides into their skin secretions. The distribution of dermal antimicrobial peptides among anuran families is sporadic.

Archaeobatrachia is thought to be a more ancient group than Neobatrachia. It consists of six families: Leiopelmatidae, Alytidae, Bombinatoridae, Pipidae (among which AMPs were found) and Pelobatidae, Scaphiopodidae (for which the presence of AMPs is uncertain) [15].

For the Leiopelmatidae family the presence of ascaphins is typical. Eight proteins with antimicrobial activity (ascaphin 1-8) were extracted from the skin of Ascaphus truei Stejneger, 1899. These proteins are structurally similar to each other, indicating possible multiple duplications of the ancestral gene. Ascaphins have a broad spectrum of antimicrobial activity when they are under intense exposure of gram-negative species [6]. It was shown that askafin-8 is a potentially important antiinfective agent that can easily be mass produsedd. This peptide showed a relatively high inhibition of growth strains of Escherichia coli Escherich, 1885 and Klebsiella pneumonia (Schroeter 1886) Trevisan, 1887 (minimum inhibitory concentration: the lowest concentration of a chemical that prevents visible growth of a bacterium, MIC < 25 μM (25 × 10–3 mol/m3)). It also has a high toxicity against mammalian cells (LC50 (the concentrations of the chemical that kills 50 % of the test animals during the observation period) against human erythrocytes = 55 μM) [16].

Among the Neobatrachia group cationic AMPs were found in the skin of the families Dicroglossidae, Hylidae, Hyperoliidae, Leptodactylidae, Myobatrachidae, and Ranidae. For the families Bufonidae, Ceratophyridae, Eleutherodactylidae, Microhylidae, Pyxicephalidae and Rhacophoridae such proteins are still not found. However, from the skin of Rhacophorus schlegelii Gunther, 1858 (Rhacophoridae) histone H2B has been extracted, which has cytolytic properties and in skin glands secret of Dyscophus guineti Grandidier, 1875 (Microhylidae) a Kunitz-type protease inhibitor with weak antimicrobial activity was found. However, it is unlikely that these substances play an important role in protecting the body [15].

AMPs families. From the skin secretions of Alytes obstetricans Laurenti, 1768 two families of structurally similar C-terminal a-amidated AMPs were allocated [15]. These proteins belong to the families alyteserin-1 and alyteserin-2. Representatives of the alisteryn-1 family show selective antibacterial activity (colonies growth inhibition) on Gram-negative bacteria and low hemolytic activity against human erythrocytes. Alysterin-1s has also been very active against nosocomial pathogens Acinetobacter baumannii Brisou & Prevot, 1954 [17]. Proteins from the alysterin-2 group primarily inhibit the growth of Gram-positive bacteria, such as Staphylococcus aureus Rosenbach, 1884, and like alysterin-1 exhibit low hemolytic activity [15].

Bombinins. All bombinins can be divided into two families according to their structural similarity: bombinins and bombinins H. These AMPs were extracted from the skin secretions of Bombinatoridae (mostly from Bombina bombina Linnaeus, 1761, Bombina variegata Linnaeus, 1758, Bombina orientalis Boulenger, 1890 and Bombina maxima Boulenger, 1905).

Bombinins were the first discovered AMPs (from the skin of B. bombina) and they have strong hemolytic properties. Later orthologs of bombinin were found, they were isolated from B. variegata and B. orientalis. These orthologs were called maximin and were later renamed to bombinin-like peptides (BLP).

Subsequently C-terminal alpha-amideted proteins of 20 amino acids were isolated, which were given the name bombinins H. Bombinins H3, H4 (B. variegata) and H6 (B. orientalis) were found later and distinguished by the presence of D-alloisoleucine (rather than genetically determined L-isoleucine) and D-leucine (instead of L-leucine) in the second position [18].

Bombinin-like proteins BLP-1 and BLP-3 showed a significant, broad antimicrobial activity against Gram-positive and Gram-negative strains and against opportunistic pathogenic Candida albicans (C. P. Robin) Berkhout, 1923. They are characterized by low hemolytic activity, while bombinins H2 and H4 are less active against bacteria but have significant hemolytic activity. Interestingly, the analogs containing D-amino acids showed better results in the destruction of bacteria than those with L-amino acids. Antimicrobial activity of hydrophobic bombinins H6 and H7 were lower except for their effect on Aeromonas hydrophilia Chester, 1901, which is common among frogs and causes the deadly red legs disease [18].

Mahainins. Xenopus laevis Daudin, 1802 was the first species, from the skin of which AMP (mahainin-1 and -2) was extracted and classified. The following analysis of skin secretions of X. laevis led to isolation and characterisation of peptide leucine-glycine amide (PGLa) and related AMPs with different activities, which are formed by post-translational processing of caerulein and xenopsin precursors. Comparing the amino acid sequences of procaerulein, promagainin and proxenopsin predicted by the nucleotide sequences of their cDNA revealed significant structural similarity in the N-terminal regions. It is suggested that the proteins may originate from a common ancestral gene that has undergone a series of duplications. Orthologs of magayinin-1 and -2, PGLa, caerulein-precursor fragment (CPF) and a xenopsin-precursor fragment (XPF) were found in secretions of skin glands of Xenopus borealis Parker, 1936 and Xenopus amieti Kobel, du Pasquier, Fischberg & Gloor, 1980 [17].

From the secretions of Xenopus tropicalis Gray, 1864 seven protective proteins (XT-1 – CT-7) were extracted, each of which are an ortholog of previously found peptide. So, XT-1, XT-6 and XT-7 are orthologs of CPF; XT-2, XT-3 and XT-4 are orthologs of XPF; XT-5 is an ortholog of PGLa. It was shown that the evolutionary pressure on the primary structure of clawed frogs AMPs was not strong enough to stabilize them, so the sequence of procaerulein- and proxenopsin-like proteins are quite variable.

Uperins and signiferins. AMPs were found in the representatives of Uperoleia and Crinia. From the skin of Uperoleia mjoberii Andersson, 1913 and Uperoleia inundata Tyler, Davies & Martin, 1981 structurally similar proteins called uperins, which were active against Gram-positive bacteria, were allocated. In Crinia species two proteins that differ by only one amino acid, called signiferin 2.1 and signiferin 2.2, which have shown an activity against Gram-positive bacteria, were found. Riparin 2.1, a protein similar to signiferin, was isolated in Crinia riparia Littlejohn et Martin, 1965 [15].

Dermaseptin superfamily. Skin secretions from the genus Hyla contain genetically closely related but structurally different AMPs, grouped in the dermaseptin superfamily (sensu stricto), which includes dermaseptins, phylloseptins, plasticins, dermatoxins, phylloxins, hyposins, caerins, and aureins.

The family was the subject of intense research because of the extraordinary occurrence of AMPs among Hylidae. Members of different groups differ in their structures and biological effects, but conservative amino acid sequences of the signal protein and the N-terminal proregione precursors of these proteins suggests that they are evolutionarily related [19].

Dermaseptins were studied in detail and were extracted from the skin of a large number of species (Phyllomedusa sauvagii Boulenger, 1882, Phyllomedusa bicolor Boddaert, 1772, Phyllomedusa oreades Brandao, 2002, Phyllomedusa distincta Lutz, 1950, Phyllomedusa hypochondrialis Daudin, 1800, Pachymedusa dacnicolor Cope, 1864, Agalychnis annae Duellman, 1963, Agalychnis callidryas Cope, 1862, and Hylomantis lemur Boulenger, 1882). The common features of all dermaseptins are the presence of residue Trp at position 3 and the constant sequence (Ala-Ala-Xaa-Lys-Ala-Ala-Leu-Xaa-Ala) in the center of the molecule. The greatest differences between these substances are in their cytolytic activity. Dermaseptins -S1, -S3, and -S5 (P. sauvagii) have a broad spectrum of antimicrobial activity (against Gram-positive and Gram-negative bacteria) and low hemolytic activity, while dermaseptin-S4 shows a strong antimicrobial and hemolytic action [20].

Plasticines are characterized by the presence of many copies of GXXXG motive and high conformational variability. For example, plasticin-DA1 can obtain an alpha-helical conformation (when bound to anionic phospholipid 1,2-dymiristoyilfosfatydylglycerol vesicles) and a beta-folded structure (when bound to zvitterionic dimirystoilofosfatydylocholin vesicles). Plasticines can be divided into different classes based on their ciytolytic activity: plasticins B1 and plasticins S1 (strongly cationic, a large number of lysine residues, broad spectrum antimicrobial activity and ability to lyse erythrocytes) and plasticins – A1, – C1, – C2, – DA1 (weakly cationic or neutral, just hemolytic effect). Proteins of Litoria genus divided into 5 groups: aureins, caerins, citropins, dahleins and maculatins [2].

Among the different kinds of proteins of the subfamilies (Phyllomedusinae and Pelodryadinae) of the Hyla family there are significant differences, but the amino acid sequence of the signal protein and N-terminal proregione of precursors of aureins and dahleyins are very similar, which may indicate a common evolutionary origin. Not all members of the subfamily Hylinae synthesise AMPs: from the skin extract of Pseudis paradoxa Linnaeus, 1758 four similar peptides, pseudin 1–4 have been allocated, although they were not allocated through the norepinefrin stimulation.

Pseudin-2 showed the highest activity against Gram-negative bacteria yet was weakly hemolytic. The peptide had a bactericidal effect on E. coli (Gram-negative) and was bacteriostatic on S. aureus (Gram-positive). As for the Hylinae family AMPs were detected in the Hyla, Hypsiboas and Osteopilus subfamilies. The only exception is Hyla punctata Daudin, 1802, skin of which has a cationic alpha-helical hylaseptin P1 (broad spectrum antimicrobial activity and low cytotoxicity on mammalian cells) and Hypsiboas raniceps Cope, 1862, with raniseptin secretion (AMP family with weak hemolytic action and structural similarity with dermaseptins) [21].

Ocellatins. All studied species of the genus Leptodactylidae (Leptodactylus fallax Müller, 1926, Leptodactylus ocellatus Linnaeus, 1758, Leptodactylus laticeps Boulenger, 1918, Leptodactylus pentadactylus Laurenti, 1968, Leptodactylus syphax Bokermann, 1969, Leptodactylus validus, Garman, 1888) produce one or more related protein, which were given names according to the name of a species: for example fallaxin, pentadactylin. Since this nomenclature does not allow us to define the evolutionary relationship of these proteins, all members of the protein family were named ocellatins titled after the first identified peptide. Ocellatins inhibit the growth of Gram-negative colonies but their action is not expressed or is very weak on Gram-positive organisms, for example yeast or C. albicans. Their hemolytic activity is very low. From the skin of L. laticeps and L. pentadactylus glycine and leucine-rich proteins, structurally similar to plastitcins, were allocated (which were previously found only in Phyllomedusa genus from the family Hylidae). They take random helical conformation in water, beta-folded conformation in methanol and alpha-helical conformation in 50 % trifluoroethanol-water. The protein from L. laticeps did not show antimicrobal actions and the peptide from L. pentadactylus, despite its weak cationicity is active against Gram-negative bacteria [15].

Kassinatuerins. In the skin of Kassina senegalensis (Hyperoliidae family) Dumeril & Birbon, 1841 kassinatuerin-1 was found, and it contained 21 amino acid residues. It has a significant inhibitory effect on E. coli and S. aureus (MIC < 10 μM) and was strongly hemolytic. Structurally similar kassinateurin-2 revealed no antimicrobial activity, although its orthologs from the secret of Kassina maculata Dumeril, 1953 were active against S. aureus [15].

Tigerinins. Tigerinins were found in the subfamily Dicroglossinae, Dicroglossidae family. These four small (11-12 amino acids), structurally similar C-terminal alpha-amidated proteins have a broad spectrum antimicrobial activity. From the skin of the representatives of the families Hoplobatrachus and Fejervarya tigerinin-like proteins that are C-terminal alpha-amidated have been allocated. They, as has been shown later, are an important factor in antimicrobial activity [22].

Rana-box containing proteins. The largest number of antimicrobial proteins has been isolated from the skin of frogs of the genus Rana. Proteins of ranids have 10-47 amino acid residues and contain a special structure, the rana-box, which is a 7-membered loop with a disulfide bridge at the C-terminus. Numerous of protective proteins of ranids are assigned to 14 families based on the similarity of their sequences. AMPs diversify within a large family and were the subject of many survey studies. Today these families of ranids AMPs are isolated: brevinin-1, brevinin-2, esculentin-1, esculentin-2, ranatuerin-1, palustrin-2, nigro-

cin-2 containing a part of the 7-membered ring; ranatuerin-2 which has a 6-membered ring; japonicin-2 which has an 8-membered ring; ranacyclins which have an 11-membered ring; C-terminal alpha-amidated temporins which are completely circular. Peptides of the brevinin-1 and temporin families have the widest distribution, being found in the majority of Eurasian and New World species. Esculentin-1, esculentin-2, and palustrin-2 have a more restricted distribution in both Eurasian and North American species. To date, peptides of the brevinin-2 family have been found only in Eurasian frogs, but brevinin-2-related peptides, lacking the C-terminal cyclic domain, have been isolated from North American ranids. Peptides of the ranatuerin-1 family have been identified only in the skins of North American bullfrogs of the Aquarana species group. Ranatuerin-2 peptides are found in most New World species, but the distribution in Eurasian frogs is much more restricted. Japonicin-1, japonicin-2, and nigrocin-2 have only been found in Asian species [23].

Brevinins-1 have a broad spectrum of antimicrobial activity and are potent hemolytic. Temporins are potential antiinfective agents due to their small size (8-21 amino acid residues) and the simplicity of the artificial synthesis. Most of them only act against Gram-positive organisms, but temporin-L (Rana temporaria, Linnaeus, 1758) and temporin-1DRa (Rana draytonii Baird & Girard, 1852) are capable of inhibiting Gram-negative species [15].

Use of AMP in phylogeny. Host defencive proteins from the skin of amphibians can be used as taxonomic and phylogenetic markers to study the evolution of different families of frogs. Studies confirm that cladistical analysis based on the primary structure of the HDPs of ranids can be used as a complement to analysis based on morphological characteristics and comparing nucleotide sequences of mitochondrial and nuclear genes.

One study, however, shows that even within the same family, species belonging to the same genus can synthesize AMPs, while other species can not. Despite the fact that the skin of amphibians from genuses Xenopus and Silurana produces a lot of AMPs, they were not found in the skin of Pipa pipa, which also belongs to the family Pipidae [15].

Use of AMP in pharmacology. AMPs also have different pharmacological effects including cardiotoxic, miotoxic and neurotoxic activity [24]. Due to the widespread use of antibiotics in recent decades, we now have an increase in the number of viruses and bacteria that are resistant to all current antibiotics. This creates a global public health crisis and the urgent need for new antimicrobial agents. Therefore, it is interesting that maculatin and caerin show strong antiviral effect and the ability to prevent the development of HIV by breaking the shell of the virion [25, 26].

Although magainin-2 has only a low activity against Gram-positive bacteria, its counterpart pexiganan with the replacement of lysine, has a broad spectrum of antimicrobial activity and a low toxicity on mammalian cells and has been studied as a therapeutic agent and particularly important against the foot ulcer in diabetic patients. Its mechanism of action is based on the formation of pores. CPF-like protein XT-7 showed moderate hemolytic activity on human red blood cells (LD50 (the amount of a material, given all at once, which causes the death of 50 % of a group of test animals) = 140 μM), limiting its use as a medicine. However, its counterpart [G4K] XT-7 is not hemolytic (LC50 = 500 μM) and retains a high antimicrobial activity. Proton magnetic resonance spectroscopy showed the decrease of analogues toxicity correlated with the decrease of helicity and increase of cationicity.

Among currently surveyed AMPs, esculentin-2LSa is the most suitable for the production of therapeutically important anti-infective agents. Its high activity (MIC = 4 μM) against S. aureus gives cause to develop a non-toxic analog with appropriate pharmacokinetic properties for the treatment of methicillin-resistant strains of S. aureus (MRSA) based on esculentin-2LSa. Eskulentin-2 shows immunomodulatory and anti-tumor properties [27].

Discussion. It is difficult now to draw a definitive conclusion regarding the importance of frog skin peptides in defending the host. It is suggested that AMPs offer definite evolutionary advantage to anurans, but their presence in skin secretions is not essential to survival. It is highly probable that AMPs in the skin do represent a component of the system of innate immunity in the limited number of species that produce them. Besides, these peptides are a potentially valuable source for manufacturing of antibiotics for human due to their antimicrobial properties. All in all, use of AMP in phylogeny and pharmacology make them important substances to explore.

Acknowledgements. Special thanks to R. J. Teschner (University of Konstanz, Germany) for help with ukrainian-english translation.

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Надійшла до редколегії 12.09.2018
Отримано виправлений варіант 15.10.2018
Підписано до друку 15.10.2018

Received in the editorial 12.09.2018
Received a revised version on 15.10.2018
Signed in the press on 15.10.2018

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РІЗНОМАНІТТЯ І ВЛАСТИВОСТІ ЗАХИСНИХ ПЕПТИДІВ ІЗ ШКІРНИХ СЕКРЕТІВ БЕЗХВОСТИХ АМФІБІЙ

Катіонні протимікробні білки є важливою частиною вродженого неспецифічного імунітету, першого захисного бар'єру, властивого майже всім живим організмам. Основним завданням таких білків є знищення небезпечних мікроорганізмів (грибків, бактерій, вірусів, паразитів і т. д.). Шкіра амфібій є багатим джерелом таких сполук, що містять відповідні захисні пептидні молекули, які виділяються і депонуються в розміщених на дорзальному боці тіла шкірних залозах. На сьогодні захисні білки були виявлені в усіх родинах земноводних, що вказує на можливість використання зв'язку між структурою цих білків та частин генома, відповідальних за їх виробництво, в еволюційних дослідженнях і пошуку філогенетичних зв'язків між окремими групами амфібій. Крім того, антимікробні білки можуть використовуватися в сучасній медицині. Амфібії можуть стати багатим джерелом біологічно активних речовин, використання яких може дати новий виток розвитку фармацевтичної промисловості. У цих речовин було відкрито набагато більше можливостей, ніж вважалося раніше. Наприклад, вони можуть використовуватися як коагулянти або як компоненти противірусної терапії. Таким чином, вивчення властивостей антимікробних білків в Україні (з 15 видів безхвостих земноводних, що належать до 5 родин (Pelobatidae, Hylidae, Bufonidae, Ranidae і Bombinatoridae) є дуже перспективним і багатообіцяючим напрямом спільних досліджень у галузях біохімії та зоології. У цій статті описуються хімічні особливості і властивості протимікробних білків, відомих зараз з літератури, і їх присутності в різних сімействах безхвостих земноводних. Основна мета роботи – показати мінливість цих речовин у амфібій, щоб дати основу для подальших досліджень антимікробних білків в Україні та вивчення їхнього фармацевтичного потенціалу.

Ключові слова: антимікробні пептиди; амфібії; захисні пептиди; шкірні залози; секрети.

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РАЗНООБРАЗИЕ И СВОЙСТВА ЗАЩИТНЫХ ПЕПТИДОВ ИЗ КОЖНЫХ СЕКРЕТОВ БЕСХВОСТЫХ АМФИБИЙ

Катионные противомикробные белки являются важной частью врожденного неспецифического иммунитета, первого защитного барьера, присущего почти всем живым организмам. Основной задачей таких белков является уничтожение опасных микроорганизмов (грибков, бактерий, вирусов, паразитов и т. д.). Кожа амфибий является богатым источником соединений, содержащих такие молекулы, которые производятся и депонируются в расположенных на дорзальной стороне тела кожных железах. В настоящее время защитные белки были обнаружены у всех семейств земноводных, что указывает на возможность использования связи между структурой этих белков, частей генома, ответственных за их производство в эволюционных исследованиях, и поиске филогенетических связей между отдельными группами амфибий. Более того, антимикробные белки могут использоваться в современной медицине. Амфибии могут стать богатым источником биологически активных веществ, использование которых может дать новый виток фармацевтической промышленности. У этих веществ было открыто гораздо больше возможностей, чем считалось ранее. Например, они могут использоваться в качестве коагулянтов или как компоненты противовирусной терапии. Таким образом, изучение свойств антимикробных белков в Украине (из 15 видов бесхвостых земноводных, относящихся к 5 семействам – Pelobatidae, Hylidae, Bufonidae, Ranidae и Bombinatoridae) является очень перспективным и многообещающим направлением совместных исследований в областях биохимии и зоологии. В этой статье описываются химические особенности и свойства противомикробных белков, известных в настоящее время из литературы, и их присутствия в разных семействах бесхвостых земноводных. Основная цель работы – показать изменчивость этих веществ у амфибий, чтобы создать основу для дальнейших исследований антимикробных белков в Украине и изучения их фармацевтического потенциала.

Ключевые слова: антимикробные пептиды; амфибии; защитные пептиды; кожные железы; секреты.

УДК 616-056.52

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МОРФОГЕНЕЗ ПІДШЛУНКОВОЇ ЗАЛОЗИ ЗА УМОВ ГЛУТАМАТ-ІНДУКОВАНОГО ОЖИРІННЯ: МЕХАНІЗМИ КОРИГУЮЧОЇ ДІЇ МЕЛАНІНУ

Досліджено морфогенез підшлункової залози в щурів за умов глутамат-індукованого ожиріння та оцінювання ефектів меланіну за певних умов. Дослідження здійснено на 45 новонароджених самцях щурів Wistar, розділених на 3 групи по 15 тварин у кожній. 1 група – новонародженим щурам інтактної групи підшкірно в кількості 8 мкл/г вводили фізіологічний розчин на 2–10-й день життя. 2 група – новонародженим щурам підшкірно в кількості 8 мкл/г вводили глутамат натрію (ГН) (4 мг/г) відповідно на 2–10-й день життя. 3 група щурів отримувала меланін (1 мг/кг), розчинений у воді (0,25 мл/100 г) (внутрішньошлунково, в/ш). Група II відповідно отримувала 2,5 мл/кг води (в/ш). Меланін було отримано з дріжджоподібних штамів Pseudonadsoniellabrunea(Nadsoniellanigra X1 з Української антарктичної станції. Введення меланіну починали через 4 тижні після народження та продовжували двотижневими курсами з перервами у 2 тижні. Протягом 4 місяців після народження щури перебували на звичайному харчовому раціоні. 4-місячних тварин декапітували, забирали підшлункову залозу для гістологічного та імуногістохімічного дослідження. Підшлункову залозу фіксували в 10 % формаліні, зневоднювали і заливали парафіновим воском. Парафінові ділянки 5 мкм були розрізані та забарвлені гематоксиліном та еозином. Оскільки запалення є одним із провідних механізмів ураження підшлункової залози під час ожиріння, прозапальну активацію клітин підшлункової залози аналізували імуногістохімічною оцінкою клітин CD68, експресією NF-kB та TNF-α.

Введення глутамату натрію викликало розвиток ожиріння зі збільшенням обсягу вісцерального жиру, зростанням кількості в ньому прозапальних макрофагів та підвищенням експресії NF-kB і TNFα. У підшлунковій залозі відбувалася гіперплазія інсулярного апарату, асоційована з макрофагальною інфільтрацією і підвищенням експресії COX-2. Введення меланіну запобігало порушенню морфогенезу підшлункової залози у тварин з глутамат-індукованим ожирінням, нівелюючи активацію прозапальних сигнальних шляхів.

Ключові слова: глутамат натрію, ожиріння, клітини CD68, експресія NF-kB і TNF-α, меланін.

Вступ. За висновком Всесвітньої організації охорони здоров'я (ВООЗ), ожиріння є однією з найбільш актуальних проблем у зв'язку з його високою поширеністю по всьому світу, а також його внеском у високі показники супутньої захворюваності та смертності [9]. Метаболічні порушення під час ожиріння призводять до виникнення ряду захворювань, а саме до розвитку цукрового діабету 2 типу, кардіоваскулярної патології (артеріальна гіпертензія, атеросклероз, ішемічна хвороба серця, цереброваскулярні розлади), змінопорно-рухового апарату (остеохондроз хребта та обмінно-дистрофічний поліартрит), уражень гепато-біліарної системи (дискінезія жовчного міхура, хронічний холецистит, жовчнокам'яна хвороба, неалкогольне жирове ураження печінки), злоякісних новоутворень, зокрема раку легень, молочної залози, раку тіла матки і яєчника тощо. [6, 25]. Ожиріння зменшує тривалість життя на 3–5, а інколи, у разі тяжких форм, на 15 років [29].

Ожиріння, особливо в ранньому онтогенезі, – це багатофакторний процес, який супроводжується змінами харчової поведінки, системних механізмів регуляції метаболізму [3, 25]. Це неминуче позначається не тільки на функціонуванні, а й на морфогенезі підшлункової залози (ПЗ). ПЗ має дві частини: екзокринну, що продукує широкий спектр травних ферментів, і ендокринну, яка формує 1–3 % маси залози і є джерелом панкреатичних гормонів [22, 27]. Ендокринна і екзокринна частини ПЗ розвиваються з одного ембрі-