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Conotoxins: From the Biodiversity of Gastropods to New Drugs

A. E. Fedosov[a, b, *], S. A. Moshkovskii[c], K. G. Kuznetsova[c, d], and B. M. Olivera[b]

[a]*Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Leninskii pr. 33, Moscow, 119071 Russia*
fax: +7 (495) 954-55-34; e-mail: fedosov_zool@mail.ru

[b]*Department of Biology, University of Utah, 257 South 1400 East, rm. 201, Salt Lake City, UT 84112-0840*
fax: 801-581-4668

[c]*Orekhovich Institute of Biomedical Chemistry of the Russian Academy of Medical Sciences,*
ul. Pogodinskaya 10, Moscow, 119121 Russia
fax: +7 (499) 245-08-57; e-mail: sergei.moshkovskii@ibmc.msk.ru

[d]*Biological Faculty, Lomonosov Moscow State University, Leninskie Gory 1, bld. 12, Moscow, 119991 Russia*
fax: +7 (495) 939-43-09

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Abstract—The review describes general trends in research of conotoxins, the peptide toxins isolated from sea gastropods of the genus *Conus*. It covers publications from conotoxin discovery in 1970th up to contemporary research and considers classification of conotoxins, their structural diversity and different ways of their action on molecular targets, particularly, ion channels. Special attention is paid to the applied aspect of conotoxin research especially to perspectives of the development of conotoxin based drugs. The first example of such conotoxin based drug is ziconotide an analgesic of a new generation.

Keywords: conotoxin, conopeptide, gastropod, ion channel, drug substance.

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INTRODUCTION

Conotoxins represent a group of relatively short oligopeptides isolated from the venom of sea gastropods belonging to the genus *Conus* that contain a large number of cysteine residues. Molluscs of this group inhabit shallow tropical marine waters of the Indian and Pacific Oceans and they are characterized by extreme taxonomic diversity; according to modern data the gastropod genus *Conus* includes more than 700 species and it appears that it is the largest genus of invertebrate animals [1]. The exclusive evolutionary success of the genus *Conus* is attributed to a unique hunting strategy of its members, which use a poisonous secret produced by special glands that immobilizes and kills their preys. Venoms of cone snails comprise mixtures of 50–200 individual oligopeptides each of them is characterized by specific physiological effects [2, 3].

According to some estimations [3, 4] natural diversity of conotoxins includes 50000–100000 individual oligopeptides. This estimate should be corrected for overlapping of toxin composition in closely related species of the genus *Conus*. However, results of analysis of the *Conus* venom by modern complex techniques suggest that the diversity of individual toxins in the venom of one *Conus* species may be underestimated by at least fivefold [5].

First conotoxins were isolated and characterized by more than thirty years ago [6, 7] and the number of studies on conotoxins is growing exponentially. High attention to conotoxins is explained by their exceptional physiological activity observed during administration of conotoxins to vertebrates and some invertebrates.

Most known conotoxins interact with ion channels and thus influence nerve impulse propagation and neuromuscular conduction. This group of compounds is characterized by combination of diverse mechanisms of action on ion channels of vertebrates and invertebrates with highly specific action of each particular conotoxin. This makes conotoxins a useful tool for studies of functioning of various types of ion channels exhibiting highly selective sensitivity to certain conotoxins. Thus, conotoxins are applicable for studies of structure and molecular kinetics of ion channels and their role in formation and propagation of action potentials and in synaptic transmission [8]. For example, discovery of ω-conotoxin GVIA, an antagonist of voltage-gated calcium channels, allowed its extensive use in consequent studies of ion channels functioning, that played significant role in neurophysiology [2]. On the other hand, high molecular specificity and effectiveness in low doses make conotoxins a perspective resource for pharmacology. At the present moment one conotoxin-based drug successfully passed through clinical trials and was approved by US Food and Drug

* To whom correspondence should be addressed.

Administration, six drugs are under clinical trials and more than 20 drugs are under preclinical laboratory trials [9–11].

1. FUNCTIONS OF CONOTOXINS IN MARINE MOLLUSCS OF THE GENUS *Conus*

Molluscs of the genus *Conus* are widely known due to their shells, which are very popular among collectioners. These molluscs are characterized by complex hunting mechanisms, that allowed them to become one of a few groups of marine invertebrates feeding on fish [12].

Cones remain relatively immobile during the daytime and are most active during the night hours. Adaptation to hunting actively moving marine animals required the development of mechanisms causing rapid and effective prey inactivation in ancesters of recent cones. Appearance of such mechanisms was associated with the development of a venom gland producing a cocktail of neurotoxins. Development of highly specialized venom gland was also accompanied by the specialization of other organs of the anterior part of the digestive system: significant modification of the buccal mass, proboscis and radula [13, 14]. As mollusc attacks its prey the poison gland secret penetrates into prey's tissues and causes its paralysis followed by deep muscle relaxation and in some cases death [7, 15].

In adaptive radiation of the genus *Conus* its members specialized on feeding on various objects; now they can be subdivided into three groups: vermivorous, moluscivorous and fish-hunting [16]. Evidently, venom compositions in members of these three groups differ significantly. At the same time, some individual toxins of the cone-snail venoms exhibit universal action. It was demonstrated that administration of the venom of *C. textile* (specialized on feeding on other molluscs) to vertebrates causes significant toxic effect [17], which could be attributed to secondary (protective) function of venom gland secret.

It was demonstrated earlier that some cone snails are dangerous for humans [18]. At least thirty cases were documented when contacts with cones were lethal for human and about 90% of these accidents were registered after contacts with *C. geographus*, the large fish-hunting species. This observation stimulated original interest to toxins produced by the molluscs of the genus *Conus* [19].

The genus *Conus* belongs to one of the most diverse and taxonomically complex superfamily Conoidea; according to recent estimations it includes more than 10000 species and most of them still remain undescribed [21]. Thus the diversity of the genus *Conus* is less than 10% of the total Conoidea diversity. Superfamily Conoidea was traditionally subdivided into three families: Conidae (cone-snails), Terebridae, and Turridae; the latter includes more than 90% representatives of the superfamily Conoidea. All conoideans have a venom gland, however, feeding mechanisms in Turridae are poorly investigated, while morphological data suggest that they feed in a similar ways with cone snails. Data on compositions of Turridae toxins have appeared recently and results of preliminary studies indicate that these toxins significantly differ from neurotoxins of cone-snails [22–24].

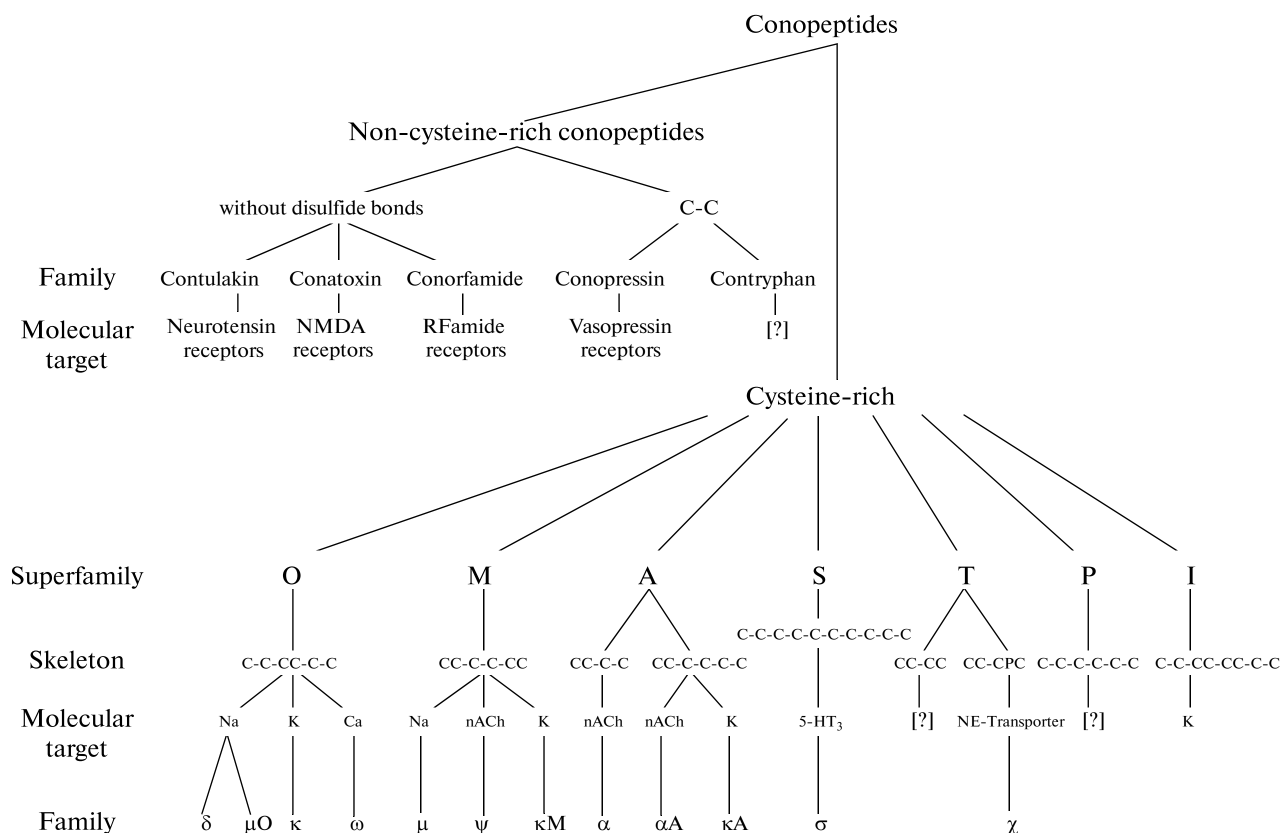
2. CHEMICAL STRUCTURE AND CLASSIFICATION OF CONOPEPTIDES

Chemically, conotoxins (in a wide sense conopeptides) are oligopeptides of 12–46 amino acid residues in length that contain a large number of cysteine residues [25, 26]. The number of cysteine residues and their arrangement (*Cys* pattern) underlie chemical classification of conotoxins (figure).

Majority of known conopeptides are referred to the group of disulfide-rich peptides (traditionally this group is known as conotoxins); they are characterized by the presence of 4–8 cysteine residues separated by other (0–6) residues. Cysteine residues form numerous disulfide bonds, which determine oligopeptide conformation. An order of cysteine residues in conotoxin molecules (we will refer to it as to *cys* pattern) gives certain disulfide bond pattern so that conotoxins characterized by the same distribution of cysteine residues have similar conformations. Thus distribution of cysteine residues in conotoxin molecules usually makes it possible to predict its conformation and (in most cases) its physiological activity. Correspondingly, distribution of Cys residues in the primary structure of conotoxins was used as the basis for their subdivision into families [27].

While cysteine residues in conotoxins are conservatively arranged, the rest of oligopeptide may be highly variable in amino acid composition; this provides exceptional diversity of primary structures of conotoxins. It was demonstrated [28] that being homologous in *cys*-pattern and physiological activity toxins of two closely related *Conus* species may significantly differ in total amino acid composition. High frequency of posttranslational modification of amino acid residues is an another important feature of most conotoxins [29], which also significantly contributes to chemical diversity of conotoxins. Some of these modifications are well known (e.g. hydroxyproline, O-glycosylated serine or tryptophan [30]), while others are rather specific (6-bromotryptophan [31], γ-carboxyglutamate [32], sulfotyrosine [33]). It was also found that some conotoxins contain D-amino acids [30].

Conopeptides assigned to another large group (of disulfide-poor peptides) may contain only one pair of cysteine residues, which form (or do not form at all) one disulfide bond [2]. These toxins are less studied in terms of diversity and physiological activity than disul-



Modern classification of conopeptides (according to [2] with additions).

fide-rich conopeptides. It was shown that disulfide-poor conopeptides mainly interact with ligand-gated ion channels; however, some of them were shown to not interact with ion channels at all [34].

3. CONOPEPTIDES EXPRESSION

Studies of conotoxins biosynthesis in epithelial cells of the venom gland of molluscs of the genus *Conus* revealed that all conotoxins are synthesized on ribosomes during translation of corresponding mRNAs. The resultant protein precursor is characterized by the structural organization typical for all conotoxins. This includes the N-terminal signal sequence (pre-site), followed by an intermediate pro-fragment and a sequence corresponding to the mature toxin (always in single copy), which is located on the C-terminal end of the precursor. During maturation all conotoxins undergo the proteolytic cleavage of the precursor with removal of pre- and pro-fragments [2, 27].

Comparison of precursor structures of various conotoxins revealed that conotoxins from closely related families (i.e. possessing the same *cys*-pattern) also share highly conserved pre-fragment [2]. Based on this fact the toxin families possessing a common signal sequence have been pooled into superfamilies. Thus, in the toxin precursor the conservative sites neighbor highly variable sites (representing the mature toxin sequence). The study of cDNA clones revealed that in mRNA, encoding the pre-fragment of the conotoxin, even the third codon position was highly conserved [28].

Mechanisms of posttranslational modifications are actively investigated and the most comprehensive results have been obtained for γ-carboxyglutamate [35, 36]. The venom gland of cone snails expresses an enzyme responsible for modification of glutamate residues; this enzyme specifically binds to the pre-fragment of the toxin precursor. Their interaction causes conformational changes of the enzyme molecule, which specifically interacts with the glutamate residues and modifies it [29].

The number of toxin gene copies and their localization in genomes remain unknown but this aspect is actively investigated now. It includes whole genome sequencing, being carried out currently for *C. bullatus* and *C. consors* (P.K. Bandyopadhyay, N. Puillandre, personal communications), that will clarify issues associated with organization of genes encoding conotoxins. Studies on evolution of genes encoding conotoxins of δ family [37] and the I-superfamily [27] revealed at least four mechanisms responsible for rearrangement of these genes: specialization, duplication, recombination and focal hypermutation. Multiple

gene duplication followed by rapid divergencies of some genes resulted in high diversity of individual toxins in each *Conus* species. Corresponding to some data [38] the venom of some *Conus* species may contain at least 15 toxins of δ family, and differences in primary structures of these toxins may significantly exceed differences between homologous toxins of closely related species. According to some recent unpublished data (N. Puillandre, personal communication) the number of individual conotoxins of the A-superfamily in one *Conus* species may be as high as 200.

The study of expression of five conotoxin families in various regions of the *C. textile* venom gland revealed significant differences between distal, medial, and proximal parts of this gland [3]. Conotoxins of A-, M-, P-, and T-superfamilies were expressed preferentially in the proximal half / two third part of the venom gland, while in the cells of the distal quarter of the gland levels of mRNA corresponding to these toxins were significantly lower. Conotoxins of the O-superfamily are the only exception: they are expressed throughout all length of the venom gland with the highest mRNA concentration in the distal part of the venom gland. The most characteristic peaks, obtained in HPLC of the *C. textile* crude venom, corresponded to the group of μ-conotoxins produced in the proximal half of the venom gland and δ-conotoxin TxVIA (its intensive peak was found the medial part of the gland) [3].

Additional study of expression of protein disulfide isomerase (PDI), the main enzyme responsible for formation of disulfide bonds essential for active conformation of conotoxin molecules, revealed equally high expression of this enzyme in all parts of the *C. textile* venom gland [3].

4. CONOPEPTIDES INTERACTING WITH VOLTAGE-GATED ION CHANNELS

4.1. Structure of Voltage-Gated Ion Channels and Diversity of Their Blockers

Voltage-gated ion channels form the group of structurally similar proteins activated by membrane potential alteration [39, 40]. These proteins characterized by selective permeability for potassium, sodium, or calcium ions play the major role in generation and transmission of action potentials. A pore of the voltage-gated ion channel can be formed by the transmembrane α-subunit that consists of four structurally homologous domains (Na^+ and Ca^{2+} channels) or by four identical or different individual subunits (K^+ channels). Activation of the voltage-gated ion channels is based on conformational changes of the channel protein complex providing its selective ion permeability. Subsequent conformational changes may cause return of the channel into its initial closed state or alternatively its temporary inactivation if the protein adopts third possible conformation [40, 41].

Toxins blocking voltage-gated ion channels are widely distributed in nature. In this section we consider properties of conotoxins interacting with the α-subunit of Na^+, K^+, and Ca^{2+} channels.

Three families of conotoxins target voltage-gated sodium channels are known to date: these include μ-conotoxins (sodium channel blockers), μO-conotoxins (inhibitors of sodium channel permeability, see below) and δ-conotoxins (blocking rapid sodium channel inactivation) [2, 42]. Calcium channel antagonists are widely known due to ω-conotoxin GVIA. ω-Conotoxins block synaptic neuromuscular transmission and due to this effect they are actively used in neurophysiology [42, 43]. Potassium channel blockers, κ- and κM-conotoxins are extremely variable and therefore poorly investigated at the moment. Molecular targets of most toxins of this group also remain unknown. Interestingly, conotoxins interacting with sodium channels are highly conserved while conotoxin antagonists of potassium channels are characterized by extreme structural and genetic variability in different taxonomic groups of the genus *Conus* [2].

Conotoxins interacting with voltage-gated ion channels cause various physiological effects. μ-Conotoxins of fish-hunting species of the genus *Conus* that interact with muscle sodium channels and ω-toxins blocking presynaptic calcium channels induce paralysis of their preys followed by irreversible impairments in neuromuscular transmission [44]. At the same time some δ-conotoxins may perform protective functions and their administration to vertebrate animals causes intensive pain [44].

4.2. Conotoxin Antagonists of Na^+ Channels

Voltage-gated sodium channels are the key proteins involved in generation and propagation of the nerve impulse. Activation of sodium channels in response to changes of the transmembrane potential is a basis for generation of action potential in electrically excitable tissues. Ten isoforms of α-subunits that determine sodium channel functioning are known to date; they have been classified using the Nav1.x nomenclature [45–47]. In addition, sodium channels have been also subdivided by their sensitivity to tetrodoxin (TTX) into TTX-sensitive and TTX-insensitive. The TTX binding site, also known as site I, binds other toxins, for example, saxitoxin (STX) [48]. There are at least five sites different from site I that provide specific interaction of the channel protein complex with various physiologically active ligands [49, 50].

4.2.1. μ-Conotoxins. μ-Conotoxins belong to the M-superfamily of conotoxins; originally, they have been isolated from the venoms of the piscivorous species *C. geographus* [51]. These oligopeptides consist of 22–25 residues and contain 6 cysteine residues form-

ing the Cys-pattern III (see figure). μ-Conotoxins interact with site I (TTX binding site) of sodium channels and block sodium current into cells. At the moment μ-conotoxins are the only known peptide ligands interacting with this site of sodium channels [2].

μ-Conotoxins GIIIA and GIIIB isolated from the *C. geographus* venom are currently the best characterized μ-conotoxins. These toxins selectively block skeletal muscle sodium channels (Nav1.4); they demonstrate significantly lower affinity to other types of sodium channels [52, 53]. Structure-functional studies revealed complex interaction of GIIIA and GIIIB molecules with the sodium channel pore, with several amino acid residues of these conotoxins involved. It was demonstrated that the GIIIA/GIIIB binding site overlaps with the TTX binding site, but is not identical to it [54, 55]. Some point mutations causing significant changes in Nav1.4 sensitivity to TTX insignificantly influenced the IC_{50} values for GIIIA and GIIIB (concentrations required for half-maximal inhibition) [56].

Numerous molecular modeling studies proposed a detailed mechanism of interaction of GIIIA molecules with the ion channel protein complex [57]. It was shown that in GIIIA molecule Arg-13 plays a key role in the blockade of the ion channel pore, acting as the steric and electrostatic barrier blocking the sodium channel pore [53, 55, 57, 58]. The GIIIA and GIIIB analogues with Arg-13 residue artificially substituted by other amino-acid demonstrated partial blockade of Nav1.4 conductivity, possibly, due to partial physical occlusion of the sodium channel pore. Usage of such analogues allowed investigation of other residue's roles in interaction between conotoxin and the channel protein complex [59].

The other well characterized μ-conotoxin is PIIIA isolated from the venom gland secret of *Conus purpurascens*. This oligopeptide also contains arginine residues (Arg-14) functionally homologous to μ-GIIIA Arg-13. PIIIA shows the highest specificity to the Nav1.4 channels in interaction with mammalian recombinant sodium channels, however in submicromolar concentrations PIIIA also blocked neuronal Nav1.2 channels [60]. This feature differs PIIIA from the μ-conotoxins GIIIA and GIIIB.

Structural studies of PIIIA have shown that in solutions this oligopeptide adopts two alternative conformations corresponding to *cis*- and *trans*-conformations of hydroxyproline-8 [61]. At *trans*-conformations of hydroxyproline-8 the three-dimensional structure of PIIIA significantly differs from the structures of GIIIA and GIIIB and this may determine differences in their interactions with sodium channels.

A recently described μ-conotoxin KIIIA is characterized by even more universal action on TTX-sensitive sodium channels [62]. In experiments on mouse peripheral neurons this toxin irreversibly inhibited 80% of TTX-sensitive and 20% of TTX-insensitive sodium channels. Administration of micromolar doses of this toxin to mice decreased pain effects induced by subsequent administration of formalin.

Recently, a novel μ-conotoxin, SmIIIA, isolated from *C. stercusmuscarium* has been shown to exhibit significant specificity in the interaction with neuronal voltage-gated sodium channels. A unique feature of SmIIIA consists in irreversible inhibition of most TTX-insensitive voltage-gated sodium channels in frog dissociated sympathetic neurons and dorsal root ganglia [60]. At the same time SmIIIA did not affect TTX-sensitive sodium channels of these neurons. Although possible effects of this novel μ-conotoxin on mammalian ion channels remain unknown its affinity to TTX-insensitive channels significantly differs this toxin from all earlier characterized μ-conotoxins.

At least 7 μ-conotoxins inhibiting amphibian TTX-insensitive sodium channels [63] and two μ-conotoxins interacting with mammalian sodium channels [64] are known to date.

4.2.2. μO-Conotoxins and δ-conotoxins. μO-Conotoxins and δ-conotoxins are hydrophobic oligopeptides that belong to the O-superfamily (figure). Although both μO-conotoxins and δ-conotoxins inhibit the permeability of sodium channels it appears that their physiological effects are being realized via a principally different molecular mechanism. It was demonstrated that in contrast to μ-conotoxins the μO-conotoxins do not compete with saxitoxin in their interaction with the ion channel protein complex and therefore do not bind to site I [48, 65]. However, exact mechanism of μO-conotoxin interaction with sodium channels remains unknown. Two closely related toxins, μO-MrVIA and μO-MrVIB consisting of 31 residues each have been isolated from the venom of the molluscivorous *Conus marmoreus*. Both toxins are characterized by the *Cys*-pattern, which is more typical for ω-toxins rather than for μ-conotoxins.

Conotoxins μO-MrVIA and μO-MrVIB can inhibit molluscan, amphibian [66], and mammalian [44, 66–70] neuronal voltage-gated sodium channels. These toxins inhibit rat neuronal TTX-insensitive sodium channels Nav.8 more effectively than TTX-sensitive ones [67, 68]. Moreover, μO-MrVIB effectively inhibited human neuronal TTX-insensitive Nav1.8 channels but showed no effect on TTX-insensitive Nav1.9 channels [68]. μO-MrVIB also inhibited the voltage-dependent sodium channels Nav1.2 and Nav1.4 and exhibited higher affinity to the channels of latter type [71]. Although the exact mechanism of μO-MrVIA and μO-MrVIB interaction with the channel protein complex remains unknown the existing data suggest that these toxins bind to the C-terminal loop of domain III of the sodium channel α-subunit [44, 71].

δ-Conotoxins are characterized by the same Cys-pattern as μO- and ω-conotoxins and they also belong to the O-superfamily. The main effect of δ-family

conotoxins consists in inhibition of rapid inactivation of voltage-gated sodium channels (the main mechanism determining shape and duration of action potentials) [44]. Inactivation of these channels results in prolongation of action potential, depolarization of cell membrane, and eventually causes massive electric overexcitation of the whole nervous system. Molecular mechanisms of δ-conotoxins are actively investigated, and it is suggested that δ-conotoxin binds to the extracellular part of the ion channel that causes conformational changes of its intracellular domain responsible for channel inactivation [2].

The observed effects of δ-conotoxins significantly depend on testing systems. Conotoxin δ-TxVIA obtained from the venom of the molluscivorous *C. textile* prolonged sodium ion current through molluscan neuronal membranes. In vertebrate systems this toxin also was shown to bind to sodium channels but without any toxic effect [72]. Another member of this group, δ-PVIA isolated from the venom of the fish-hunting species *C. purpurascens* caused clear symptoms of nerve system overexcitation in fishes and mice, but it was basically ineffective in molluscs even at the dose much (100-fold) higher than the dose effective in fishes. The conotoxins δ-PVIA and δ-SVIE inhibited mammalian voltage-gated channels [73]. Another δ-conotoxin, EVIA prevented inactivation of the sodium channels Nav1.2, Nav1.3, and Nav.1.6 in rat neurons, but did not interact with the rat muscle channel Nav1.4 and did not influence the human myocardial voltage-dependent sodium channel Nav1.5 [74]. Interestingly, conotoxins δ-PVIA and δ-GmVIA may block the sodium channels Nav1.2 and Nav1.4 expressed in *Xenopus* oocytes [70], but the mechanism of their interaction with protein complexes of these channels is similar to the mechanism of μO-conotoxin interaction [44, 71].

Recent studies of physiological activity of conotoxins of the I-superfamily [75, 76] isolated from the piscivorous species *C. radiatus* revealed high affinity of these toxins (iRXIA) to the Nav1.6 channels. It was also found [77], that the modified residue D-Phe-44 of the 46-mer oligopeptide iRXIA plays a key role in the oligopeptide interaction with the sodium channels. The conotoxin iRXIA isolated from the *C. radiatus* venom gland and its synthetic analogue demonstrated the same effect: generation of repeated action potentials on membranes of tested cells. The synthetic analogue of iRXIA containing L-Phe-44 did not cause electrophysiological activity in tested cells even at concentrations, which were 10–20 fold higher than the acting concentrations of iRXIA [4].

4.3. Conotoxins—Antagonists of K^+ Channels

Potassium channels play an important physiological role by providing membrane repolarization after action potential and have a number of other functions in different cell types [78]. According to the wide spectrum of physiological functions several families of voltage gated potassium channels have been recognized (Kv1.x, Kv2.x, etc.); their proteins are encoded by more than 80 genes. The α-subunit of the voltage gated potassium channels contains six transmembrane domains, the functional protein complex that form the channel pore may be homo- or heteromeric [79]. Although the first conotoxins interacting with potassium channels have been discovered and characterized by 1996 [80] only some of them have been studied in details. Studies of molecular mechanisms of conotoxin interaction with heteromeric K^+ channels require significant efforts due to complex structure of the channel, which significantly complicates identification of particular molecular targets of the conotoxin [25].

Recent studies have shown that various taxonomic groups of cone snails produce several families of toxins interacting with the potassium ion channels [81, 82]; however exact physiological effects and their molecular specificity especially in mammalian expression systems remain poorly investigated.

4.3.1. κ-Conotoxins. κ-Conotoxin PVIIA obtained from the venom of the piscivorous cone *C. purpurascence* was the first conotoxin that demonstrated active interaction with voltage gated potassium channels [60, 80]. Experiments on Shaker drosophila potassium channels [83] cloned into *Xenopus* oocytes revealed highly effective inhibition of potassium current through these channels by κ-PVIIA (IC_{50} of 50 nM) [2]. However, it appears that the tested Shaker channels are not the natural molecular target for PVIIA. The target has not been identified yet. Although κ-PVIIA administration caused clear symptoms of excitement in mice, none of more than 20 types of cloned potassium channels tested exhibited specific interaction with this conotoxin. Nevertheless, it was demonstrated that κM-conotoxin RIIIK can block mouse Kv1.2 [84]. This oligopeptide also demonstrated a cardioprotective effects in the mouse model of ischemia [85, 86].

Evidently, blockade of potassium channels by conotoxin κ-PVIIA plays an important role in hunting of piscivorous cone snails: κ-PVIIA plays a key role in rapid immobilization of the prey [2]. Physiologically κ-PVIIA acts synergistically with δ-conotoxin PVIA; this results in general overexcitation of membranes of electro-sensitive cells of the prey followed by its almost immediate titanic paralysis [25].

Interaction of κ-PVIIA with the potassium channel protein complex can be characterized by bimolecular mode. The analysis of Shaker channels blocked by κ-PVIIA has shown that the conotoxin binds to the external side of the channel pore. Artificial Shaker channels carrying mutation in the P-loop were characterized by significantly lower affinity to κ-PVIIA [60]. Subsequent studies revealed that κ-PVIIA as well as other potassium channel antagonists contains a

characteristic motif (Lys-7 and Phe-9) playing a key role in potassium channel blockade [87].

These results support "the critical dyad model" proposed [88, 89] for protein antagonists of potassium channels. The primary structure of κ-PVIIA doesn't show significant homology with protein antagonists of K^+ channels of other poisonous animals. It appears that their common functional elements providing interaction with ion channels underwent convergent development. Thus, various potassium channel protein antagonists possessing a common key amino acid motif and isolated from various groups of poisonous animals may have common or similar mechanisms of interaction with ion channels regardless of their cysteine backbone.

4.3.2. κA- and κM-conotoxins. κA- and κM-conotoxins significantly differ structurally. κA-Conotoxins are O-glycosylated oligopeptides that belong to the A-superfamily [25], while κM-conotoxins belong to the M-superfamily and their disulfide bond pattern is close to μ-conotoxins [81].

The first κA-conotoxin, κA-SIVA, was isolated from the venom of the piscivorous species *C. striatus*. Administration of this conotoxin to mice caused spastic paralytic symptoms [30]. Electrophysiological tests revealed that κA-SIVA caused repeated excitation in frog *Musculus cutaneous pectoris* and sympathetic ganglion neurons. Micromolar concentrations of κA-SIVA blocked the Shaker potassium channels expressed in *Xenopus* oocytes. However, molecular mechanisms underlying high affinity of κA-SIVA to vertebrate potassium channels remain unknown. Interestingly, κA-SIVA contains O-glycosylated Ser-7 and κA-conotoxins are the only known conopeptides characterized by posttranslational O-glycosylation [30].

Studies on another toxin, CcTx isolated from *C. consors*, which shares a common Cys-pattern with κA-SIVA suggest that this oligopeptide activates voltage-gated potassium channels of resting cells [90]. If these results are confirmed elsewhere this will mean that effects of structurally similar conotoxins may be realized by various molecular mechanisms.

Conotoxins of a recently found κM-family are characterized by the same cysteine pattern as μ-cotoxins and ψ-conotoxins but demonstrate different molecular specificity and exhibit high affinity to voltage-gated potassium channels [81]. The first characterized toxin of this group, κM-RIIIK, was isolated from the *C. radiatus* venom gland. In contrast to structurally related μ-conotoxins κM-RIIIK does not interact with potassium channels expressed in *Xenopus* ooxytes but effectively blocked the Shaker K^+ channels (IC_{50} of 1 μM) in the same expression system and exhibits even higher affinity to the trout TShal K^+ channels (IC_{50} of 20 nM) [25].

Interaction of this conotoxin with the channel pore is a bimolecular reaction. As in the case of κ-PVIIA amino acid substitution in the protein complex forming the Shaker channel pore significantly decreased its affinity to κM-RIIIK. It should be noted that κM-RIIIK lacks Phe or Tyr residues forming the "critical dyad" responsible for toxin interaction with the potassium channel. Structural analysis of κM-RIIIK has shown that this toxin interacts with the pore region of Shaker channels and the mechanism is close to that of μ-GIIIA than to κ-PVIIA [91].

Besides the conotoxins considered above there are at least three families of conotoxins antagonists of voltage gated potassium channels and these conotoxins significantly differ from those considered above [82]. Toxins of the I-superfamily are the most interesting ones. They may block both K^+ and Ca^{2+} voltage gated channels [25]. The study of molecular specificity of ViTx, the I-superfamily toxin, has shown that it specifically blocks mammalian Kv1.1 and Kv1.3 channels but does not interact with Kv1.2 channels [92].

A toxin of the O-superfamily isolated from the *C. monile* venom also demonstrated specific interaction with mammalian voltage gated potassium channels. This conotoxin inhibited all types of potassium channels in the rat dorsal root ganglion [93].

4.4. ω-Conotoxins—Antagonists of Ca^{2+} Channels

Voltage gated calcium channels mediate calcium influx in response to cell membrane depolarization; they are also involved in many signaling processes in living organisms, particularly in neuromuscular transmission [94]. Although the calcium channels are heteromeric protein complexes, their physiological activity is mainly determined by the transmembrane α1 subunit forming the channel pore [95].

The voltage gated calcium channels are subdivided into L-, N-, P-, Q-, R-, and T-types by differences in pharmacological and physiological characteristics they exhibit [96]. Later, they were classified according to the standard nomenclature originally developed for the potassium channels. The Cav1 family of voltage gated calcium channels corresponds to L-type, the Cav2 family includes the channels of P/Q, N-, and R-types, while the Cav3 family is formed by the type T calcium channels [97].

Most of conotoxin antagonists of calcium channels belong to the ω-family. They were among the first well characterized conotoxins and they are used most frequently in neurophysiological studies. Use of ω-conotoxins allows selective block of calcium channels of certain subtypes in studies of molecular mechanisms of synaptic transmission. As in the case of other well studied groups of conotoxins the best characterized ω-conotoxins were isolated from venoms of piscivorous species of the genus *Conus* [25]. According to results of pilot studies molluscivorous and vermivorous

cone snails also have a diverse set of conopeptides which specifically interact with Ca^{2+} channels. For example, there are conotoxins that selectively block molluscan Ca^{2+} channels, pharmacologically corresponding to mammalian L-type of calcium channels [72].

The venom gland secret of *Conus* species usually contains a set of individual ω-conotoxins, which significantly differ in their primary structure and obviously are specific to different types of Ca^{2+} channels. This was demonstrated for the venom of *C. magus*, containing two ω-conotoxins, MVIIA and MVIIC. The first one specifically interacted with the N-type of calcium channels (Cav2.2), while the second one was more selective in blockade of the P/Q channels (Cav2.1). The residue Tyr-13 played the major role in interactions of both conopeptides with the protein complex of calcium channels, while contribution of Lys-2 was less important [98, 99].

Structural studies have shown that the primary structure of ω-conotoxins is characterized by a high content of basic amino acid residues, which are crucial for inhibition of Ca^{2+} channels [100].

Conotoxin ω-TxVII contains functionally important Phe-11 and Trp-26 and it is more hydrophobic, than most known ω-conotoxins. It was suggested that ω-TxVII may interact with same sites on the surface of the calcium channels as some non-peptide inhibitors of lower molecular mass (e.g. dehydropyridines and phenylalkylamines [101]). For example, Gla-containing contryphan can also inhibit calcium channels as it was demonstrated on mammalian L-type channels [102].

5. CONOTOXINS INTERACTING WITH LIGAND GATED ION CHANNELS

5.1. Structure and Diversity of Ligand Gated Channels and Diversity of Their Blockers

Ligand gated ion channels of postsynaptic membranes play a key role in the nerve impulse propagation. One large group is formed by ligand gated ion channels activated by acetylcholine, serotonin, GABA, and glycine. All these channels share a similarity: their functional protein complexes are formed by five subunits, each of them contains four transmembrane α-helices [103]. The other large group the glutamate-gated ion channels, is usually subdivided into N-methyl-D-aspartate (NMDA) and kainate (non-NMDA, AMPA) receptors. Finally, ATP-gated channels, the third family of ligand gated ion channels, function in some synapses [104].

Conopeptides interact with all three families of the ion channels. Most of them interact with ion channels of the first family: these toxins represent one of the main *Conus* venom components. Toxins interacting with nicotinic acetylcholine receptors are the most diverse and well studied ones [105, 106]. Although noncompetitive nicotine agonists have been found in the *Conus* venom, competitive agonists are especially abundant. Conotoxin inhibitors of glutamate NMDA receptors represent unusual group of conopeptides characterized by lack of the "disulfide backbone" and large proportion of γ-carboxyglutamate residues [35].

Evidently, general effect of conotoxins inhibiting postsynaptic receptors consists in blockade of neuromuscular transmission that finally results in paralysis of the prey [107]. All conotoxins, known to inhibit acetylcholine receptors of neuromuscular type (nAChR) share this physiological effect. These toxins quantitatively dominate in the venom of piscivorous *Conus* species. Physiological effects of conotoxins acting at other types of nicotine-sensitive acetylcholine receptors are less clear. It is believed, that they act in complex with other conotoxins suppressing protective reflexes of their preys [108].

Another complex effect has been proposed for conotoxins, antagonists of glutamate-gated and 5-hydroxytryptamine type 3 (5HT3) receptors. Results of pilot studies of physiological effects of these toxins suggest that they can partially block activity of sensitive neurons that causes the nirvana-like state of their preys [109].

5.1.1. α-Conotoxins and nAChRc. Structurally, nicotine-sensitive acetylcholine ion channels are formed by protein complexes, which include five transmembrane subunits [103]. nAChRc receptors of invertebrates and some vertebrates (α7, α8, and α9 subtypes) are homomeric (and formed by five structurally identical subunits). However, most nAChRc receptors of vertebrates are heteromeric proteins that consist of two α-subunits and three other subunits. Each receptor contains two acetylcholine binding sites located at the border between α-subunits and adjacent subunits of the ion channel. Transition of the ion channel from the closed to open state requires interaction of two acetylcholine molecules with both nAChRc binding sites [110, 111].

Specific toxins competitively inhibiting acetylcholine binding sites are wide spread in nature; these include such well known neurotoxins as α-bungarotoxin or a curare alkaloid [112]. Use of these toxins substantially contributed to investigation of structure and functioning of the muscle subtype of nAChRc, however, only discovery of a diversity of α-conotoxins demonstrating narrower specificity enabled the investigation of other types of nAChRc [113]. In mammalian nicotine-sensitive ion channels one of subunits contacting with the α-subunit undergo substitution during channel formation: γ-subunit of the fetal receptor is substituted by an ε-subunit in the mature receptor [114]. This causes structural rearrangements in the acetylcholine binding site located at the border of α/γ–α/ε subunits. It was demonstrated that all ear-

lier known nAChRc inhibitors specifically bind only to a definitive form of the receptor and do not interact with fetal one. First toxins that are able to bind specifically with the mammalian nAChRc α/γ site were found in the *Conus* venom [113].

Inhibitors of all classes of nicotine-sensitive receptors described above have been found in α-conotoxins [107]. Some subfamilies of α-conotoxins have been found only in closely related *Conus* species. For example, there is a subfamily of α-3/5-conotoxins, the main venom of the piscivorous *Pionoconus* species [27]. These oligopeptides share a common primary structure CCX_3CX_5C, where X is any amino acid residues; these are paralytic toxins acting at muscle nAChRc [105]. These toxins demonstrate high specificity towards one or two acetylcholine binding sites of mammalian nAChRc. For example, MI α-conotoxin demonstrated much higher (104-fold) affinity to the α1/δ-site than to the α1/γ-site. High affinity of MI to the α1/δ-site is determined by specific interaction with amino acid residues of the δ-subunit of the ion channel [115]. Interestingly, the best characterized conotoxins of the α3/5 subfamily (MI and GI) demonstrate significantly higher specificity to muscle nAChRc than other known nAChRc inhibitors. For example, α-bungarotoxin inhibits both muscle nAChRc and α7 receptors, while curare inhibits not only muscle nAChRc but also many other known nicotine-sensitive ion channels [110].

Frequently venom of one *Conus* species contains a set of individual toxins of the α3/5 subfamily encoded by various genes. For example, the venom of the fish-hunting *C. striatus* contains conotoxins SI, SIA, and SII of the α3/5 subfamily; although they are differed in the primary structure but exhibit the same physiological effect, muscle paralysis of their preys [2]. However, effects of these conotoxins on higher vertebrate nAChRc are less predictable: some conotoxins of the α3/5 subfamily (e.g. MI and GI) are very effective inhibitors of muscle nAChRc, while others (SI) are less active [116].

The other specific subfamily of α-conotoxins, α4/3 is characterized by the primary structure with the motif, $-CCX_4CX_3C-$. These conotoxins have been found in closely related cone species feeding on polychaetes of the *Amphinomidae* family [117]. The best characterized representatives of this group are two toxins isolated from the *C. imperialis* venom, ImI and ImII. Both these toxins specifically inhibit homomeric invertebrate nicotine-gated ion channels. They also specifically inhibit vertebrate α7 receptors [117, 118]. It is suggested that all toxins of the α4/3 subfamily are specific to homomeric nAChRc.

Studies of molecular interaction of ImI with α7 receptors have shown that this toxin binds to the acetylcholine binding site and thus functions as a competitive inhibitor of α7 receptors [119–121]. Conotoxin ImII shares high structural similarity with ImI (9 of 12 amino acids of these toxins are identical) and it also inhibits the α7 subtype of nAChRc receptors. However, unexpectedly, it was demonstrated that ImII is not a competitive inhibitor of the acetylcholine binding site [122].

It was shown that Pro-5 plays the key role in the interaction of ImI with the acetylcholine binding site, while ImII contains arginine residue at this position and so ImII binds to some other (unidentified) site of the α7 subtype nAChRc. Most α-conotoxins contain proline residues and function as competitive inhibitors of nAChRc. Thus, this residue might be used as a marker of molecular mechanism responsible for α-conotoxin binding. However, structural studies revealed that only homomeric nAChRc may be inhibited by α-conotoxins non-competitively. Molecular kinetics of interaction between heteromeric nAChRc with α-conotoxins lacking proline residue currently remains unknown [122].

α-4/7 ($-CCX_4CX_7C-$) is the largest and the most widespread subfamily of α-conotoxins. Despite high specificity of each particular conotoxin the α-4/7 subfamily is generally characterized by a wide spectrum of their nicotine-sensitive acetylcholine receptor targets. For example, α-EI and members of the α-3/5 subfamily interact with muscle nAChRc, while α-PnIB interacts with homomeric α7 nAChRc and the toxins α-MII and α-AuIB inhibit neuronal nAChRc [123]. Even minor changes in the primary structure may significantly influence specificity of conotoxins of the α-4/7 subfamily. For example, sequences of conotoxins α-PnIA and α-PnIB, isolated from the *C. pennaceus*, differ by two amino acid residues and α-PnIA is a highly specific inhibitor of α3β2-nAChR, while α-PnIB inhibits homomeric α7 nAChRc. A synthetic oligopeptide obtained from α-PnIA and α-PnIB [124] demonstrated even higher affinity to α7 receptors than both toxins; after alternative fusion of the α-PnIA and α-PnIB elements, the resultant "reverse" oligopeptide was characterized by insignificant affinity to the investigated receptors.

α-Conotoxins are well described in the literature and recently several comprehensive reviews with detailed information on their diversity and mechanisms of action have been published [106, 107, 125].

5.2. Other Families of Conopeptides Interacting with Ligand-Gated Ion Channels

Besides α-conotoxins there are at least 2 families of conotoxins that interact with nicotine-sensitive acetylcholine receptors. Members of these families, αA- and ψ-conotoxins, block ion permeability of nAChRc. All investigated αA- and ψ-conotoxins interact with muscle nAChRc [126, 127].

αA-conotoxins structurally differ from α-conotoxins by the presence of three disulfide bonds (α-conotoxins have only two bonds). Nevertheless, they also

are competitive inhibitors of nAChRc, which, however, did not demonstrate high specificity towards the α1/δ acetylcholine binding site [128, 129]. αA- (as well as α-) conotoxins have been found in venoms of some closely related piscivorous *Conus* species. ψ-Conotoxins are usually detected in venoms of the same *Conus* species as αA-conotoxins. In contrast to α- and αA-conotoxins, known ψ-conotoxins do not act as competitive inhibitors of muscle nicotine-sensitive acetylcholine receptors [25].

At this moment both these families are rather poorly investigated. Primary structures of several αA- and two ψ-conotoxins have been determined and structurally ψ-conotoxins demonstrate similarity with μ- and κM-conotoxins, but molecular specificity of these toxin families significantly differs [130–133].

Serotonin (5-HT3) receptors represent another group of the ligand gated ion channels sensitive to highly specific inhibition by a conotoxins. The study of molecular specificity revealed that unusual conopeptide containing bromotryptophan residue, σ-conotoxin GVIIIA, demonstrated high affinity to 5-HT3 receptors [134]. This is the only known protein toxin characterized by interaction with 5-HT3 receptors. Molecular specificity of other conopeptides, assigned to the same superfamily as GVIIIA, remains unknown, however, taking into consideration differences in their primary structures it seems unlikely, that they interact with 5-HT3 receptors as GVIIIA does.

Conantokins, members of a distinct group of conopeptides characterized by lack of the cysteine backbone, do also interact with ligand-gated ion channels, particularly with NMDA receptors (glutamate gated channels) [7, 35].

According to current views NMDA receptors are tetramers formed by two types of subunits, NR1 and NR2 [135]. Only one gene encoding the NR1 subunit has been identified so far, while there is evidence that four structurally types of the NR2 subunit (NR2A, NR2B, NR2C, and NR2D) are encoded by four different genes [2].

Among conopeptides interacting with NMDA receptors conantokin-G is the best studied. It is a short linear oligopeptide of 17 amino-acied residues that does not have a single cysteine residue, but contains 5 residues of γ-carboxyglutamate (Gla) [25]. Gla is formed from glutamate by the vitamin K-dependent enzyme, γ-glutamyl carboxylase. Conantokin-G isolated from *Conus* venom was the first invertebrate peptide containing Gla residues. γ-Glutamyl carboxylase cloned from the *Conus* venom gland is characterized by unexpectedly high homology with mammalian γ-glutamyl carboxylase [32, 136]. It is suggested that in the absence of the cysteine backbone γ-carboxyglutamate residues play the major role in determining conformation of conantokin-G.

Interaction of NMDA with corresponding competent receptors results in activation of a cascade mechanism and synthesis of cGMP [137]. Treatment of neonatal rat cerebellums with conantokin-G revealed that subsequent addition of NMDA does not result in the increase of cGMP; at the same time treatment with conantokin-G does not influence kainate receptor functioning [138]. Studies of molecular specificity revealed that in submicromolar concentrations this toxin specifically binds to the NR2B subunit of NMDA receptors [2], but shows no effect on receptors containing the NR2A subunit even at concentration 50 μM [139].

Effects of conantokin-G on mammalian organisms are age-related. For example, administration of conantokin-G to young mice resulted in a sleep-like state, while in aged mice this toxin caused a hyperactive state [140, 141].

In later studies some other conantokins from venoms of fish-hunting *Conus* species, were isolated and characterized, these. These conantokins, (conantokin-B [142], conantokin-L [143], conantokin-P [144], conantokin-R and -T [145]) were also shown to block NMDA receptor. In studies of molecular specificity most these toxins demonstrated equally effective binding to NR2A and NR2B subunits with the only exception of conantokin-T, that did not bind to NMDA receptors containing the NR2D subunits [145].

Contulakin-G isolated from the venom gland of *Conus geographus* is the only invertebrate peptide interacting with neurotensin receptors [10]. Structurally, contulakin-G is an 16 amino acid oligopeptide containing posttranslationally α-glycosylated Trp-10. Its C-terminal part shares similarity with neurotensins. Intraventricular administration of the synthetic glycosylated contulakin-G to mice caused locomotor impairments; administration of a native contulakin-G caused the same effect. Glycosylated contulakin-G was effective at concentrations one order of magnitude lower than the nonglycosylated Thr-10 contulakin-G. Glycosylated and nonglycosylated Thr-10 contulakin-G interacted with various mammalian neurotensin receptors including human (type 1, hNTR1), rat (types 1 and 2), and mouse (type 3) receptors [29]. Physiologically both oligopeptides act as agonists of the neurotensin receptors [10].

Table 1 summarizes data on some other conopeptides interacting with various ligand-gated ion channels and cell receptors. Studies of physiological activity of these receptors are at the very beginning so that little is known about them at the moment. Molecular targets and effects of most of these conotoxins remain poorly investigated and it is suggested that they are active mainly in invertebrates [25].

Table 1. Some poorly investigated conopeptides that are not referred to the main types

Conopeptide name	*Conus* species	Molecular target or effect	Primary structure
Blockers of G-protein coupled receptors (GPCR)			
Conopressin-G	*C. geographus*	Vasopressin receptors	CFIRNCPLG
Contulakin-G	*C. geographus*	Neurotensin receptors	ZSEEGGSNFNKKPYIL
P-conotoxin TIA	*C. tulipa*	α_1-adrenergic receptors	PNWRCCLIPACRRNHKKFC
Others			
mr5a χ-Conotoxin	*C. marmoreus*	Noradrenaline transport	NGVCCGYKLCHOC
μ-PnIVA	*C. pennaceus*	Mollusc sodium channels	CCKYGWTCLLGCSPCGC
γ-Conotoxin PnVIIA	*C. pennaceus*	Mollusc pacemaker ion channels	DCTSWFGRCTVNSγCCSN-SCDQTY-CγYAFOS
Conorfamide	*C. spurius*	RFamide receptors, ENaC channels	GPMGWVPVFYRF
Contryphan-R	*C. radiatus*	Calcium activated potassium channels	GCOWEPWC
Conolysine-Mtl	*C. mustelinus*	Eukaryotic cell membranes	FHPSLWVLIPQYIQLIRKILKSG
Conolysine-Mt2			FHPSLWVLIPQYIQLIRKILKS

6. DEVELOPMENT OF CONOTOXIN-BASED DRUGS

As it has been shown above, conotoxins specifically interact with ion channels of excitable tissues and cause impairments in nerve impulse propagation in the nervous system or blockade of neuromuscular transmission. Most conotoxin-based drugs, which are under clinical trials currently, have analgesic action or may be used in neurological disorders, migraines, and epilepsy [10]. Recently it has been demonstrated that some conotoxins exhibit remarkable cardioprotective effect [11].

Use of conotoxin blockers of voltage gated ion channels μ-SIIIA, μO-MrVIB, and also ω-conotoxins MVIIA and CVID is based on blockade of synaptic transmission of affector neuron chains, they cause. The major molecular targets for these conotoxins and conotoxin-based drugs are "nerve type" sodium and calcium channels located in synaptic endings of the spinal cord. These synaptic endings are responsible for transmission of nerve impulses from peripheral nervous system in the brain causing the feelings of pain [10].

Conotoxin-based drugs have evident advantages over other analgesic preparations. In contrast to opioid analgesics conotoxin-based drugs do not cause a decrease in concentrations of specific receptors and so there is no need to correct their dosage. In contrast to morphine and other opiates they do not cause tolerance and dependence [10].

Ziconotide also known under the trade name Prialt is the best pharmacologically studied preparation. This synthetic drug is based on the structure of ω-conotoxin MVIIA, a blocker of the N-type calcium channels. It was approved by the US Food and Drug Administration (FDA) in 2004. Ziconotide is characterized by considerable analgesic effect that exceeds the morphine effect; since it employs principally different mechanism of its analgesic action it may be combined with morphine or its derivatives. Ziconotide is prescribed to treat chronic pains and it is actively used as pain reliever in patients with malignant tumors or AIDS. The pharmacological effect of ziconotide has been well described in numerous publications [10, 146].

The pharmacological effect of nicotine-sensitive acetylcholine receptor antagonists based on the structure of conotoxins α-Vc1.1 and α-RgIA is associated with blockade of α9α10 receptors of the sensory neurons. Both these conotoxins demonstrate significant analgesic action in various rat pain models. At the moment ACV-1 based on the conotoxin α-RgIA structure is at the final phase of clinical trials. NMDA receptors blocked by conotoxins play an important role in some acute and chronic neurological disorders [10]. Activated calcium channels associated NMDA-receptors are characterized by high ion permeability. Thus, their excessive activation may cause massive overexcitation and neuronal death. The study of conotoxin effects on induced intensive pain, convulsions, and post-ischemia revealed high pharmacological activity of conatokin-G, which was used for the development of CGX-1007 (Cognetix); now it is under clinical trials [147]. It may be used for medication of intensive pain and epilepsy and in addition, CGX-1007 exhibits a neuroprotective effect in the rat model of ischemia [148].

Intrathecal administration of contulakin-G caused blockade of affector neuron chains and a significant analgesic effect. Contulakin-G was used as a basis for the development of GCX-1160, which is now under clinical trials. It may be used for treatment of intensive pains associated with traumatic dysfunctions of the spinal cord [10].

Table 2. Conopeptide prototypes for the development of drug substances

Conopeptide	Pharmacological name	Prescription	Molecular mechanism/target
ω-MVII-A	Ziconotide (trademark Prialt)	Unrelieved pains, for example, in malignant tumors	N-type calcium channel blocker
ω-CVID	AM-336	Neuropathic pains	N-type calcium channel blocker
Contulakin-G	CGX-1160	Neuropathic pains	Neurotensin receptor agonist
Conantokin-G	CGX-1007	Unblockable epilepsy	NMDA receptor antagonist
χ-MrIA	Xen2174	Neuropathic pains	Noradrenaline transport inhibitor
α-Vcl.1	ACV-1	Neuropathic pains	Antagonist nAChRc
κ-PVIIA	CGX-1051	Myocardial infarction, cardioprotective effect	Potassium channel blocker
μO-MrVIB	CGX-1002	Neuropathic pains	Selective blocker of sodium channels
μ-SIIIA	PEG-SIIIA	Inflammatory pains	Sodium channel blocker
Not assigned	CGX-1204	Myorelaxant	Antagonist nAChRc

The Xen 2174 preparation developed on the basis of chemically modified χ-conotoxin Mr also exhibits the analgesic effect. It causes reversible non-competitive blockade of noradrenaline transport by a specific transporter (NET) without any effects on transport of other monoamines such as serotonin and dopamine [149]. The Xen 2174 binding site on human NET partially overlap with cocaine and antidepressant desimpramine binding sites [150]. Pharmacological studies have shown that Xen 2174 causes a significant analgesic effect in patients with malignant tumors or spinal cord injuries. Recently, Xen 2174 was reported to be safe and effective drug for medication of pain syndrome in oncologic diseases [10]. Now it is on the final phase of clinical trials.

Table 2 lists conotoxin prototypes for development of drugs.

CONCLUISONS

Conotoxins are a large group of physiologically active oligopeptides acting on various molecular targets and characterized by high physiological specificity. Coordinated effects of many individual toxins presented in the *Conus* venom play a key role in hunting and defense of these animals and venom composition of each certain *Conus* species reflects specificity of its interaction with preys, predators and possibly with competitors. Systematic studies of conopeptides revealed huge diversity of their physiological effects and partially explained mechanisms of their action. High molecular specificity and diversity of conopeptides make them an important resource for pharmaceutical industry; this resource is used for development of highly effective preparations mainly used for medication of functional disorders of the nervous system. On the other hand, conopeptides are actively used in studies of molecular bases of functioning of specific ion channels and their role in the nervous system. Studies of conopeptides indicate importance of biochemical investigation of a diversity of animal and plant organisms; this is especially important if we take into consideration modern practice of biochemical studies employing limited number of model objects.

Blind search based on combinatorial libraries of synthetic inhibitors of human receptors involved in pathogenesis of various diseases is costly and technically complex approach. One of the ways for search of drug prototypes consists in their detection (identification) in secrets and venoms of poisonous and blood sucking animals. The work on development of particular drug de novo, which researchers plan to overcome within several years, nature has been overcoming over millions of years. Thus, studies of physiologically active components in numerous animal species by modern methods of molecular biology may provide realization of translational medicine [151] in a short time. The development of the ω-conotoxin based analgesic drug ziconotide is a good example illustrating effective transfer of basic knowledge into clinical practice. Studies employing whole genome sequencing of these organisms will help to provide rapid access to structures of protein toxins and protease inhibitors. Significant scientific background for studies of secrets and venoms of animals has been created in recent years and Russian researchers make certain contribution to this field. They actively participate in studies of conotoxins [118], a salivary gland secret of a medicinal leech [152], snake [153] and arachnid [154] venoms.

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