Review

Venom-Derived Neurotoxins Targeting Nicotinic Acetylcholine Receptors

Ayaulym Bekbossynova, Albina Zharylgap and Olena Filchakova *

Biology Department, School of Sciences and Humanities, Nazarbayev University, Kabanbay Batyr Ave., 53, Nur-Sultan 010000, Kazakhstan; ayaulym.bekbossynova@nu.edu.kz (A.B.); albina.zharylgap@nu.edu.kz (A.Z.)
* Correspondence: olena.filchakova@nu.edu.kz

Abstract: Acetylcholine was the first neurotransmitter described. The receptors targeted by acetylcholine are found within organisms spanning different phyla and position themselves as very attractive targets for predation, as well as for defense. Venoms of snakes within the Elapidae family, as well as those of marine snails within the Conus genus, are particularly rich in proteins and peptides that target nicotinic acetylcholine receptors (nAChRs). Such compounds are invaluable tools for research seeking to understand the structure and function of the cholinergic system. Proteins and peptides of venomous origin targeting nAChR demonstrate high affinity and good selectivity. This review aims at providing an overview of the toxins targeting nAChRs found within venoms of different animals, as well as their activities and the structural determinants important for receptor binding.

Keywords: nAChR; α-conotoxins; three-finger α-neurotoxins

1. Introduction

1.1. Structural Features of Nicotinic AChRs for Ligand Interaction

Nicotinic acetylcholine receptors (nAChRs) are channel-coupled membrane receptors activated endogenously by acetylcholine (ACh) [1]. Together with ionotropic GABA (gamma-aminobutiric acid), glycine, 5-HT3 (5-hydroxytryptamine), and zinc activated ion channels, they belong to a cys-loop superfamily of ligand-gated ion channels [2]. The receptors are characterized by radial symmetry and have pentameric organization, with five subunits arranged radially around a central ion-conducting pore. The ACh binds at the interface between adjacent subunits. There are 17 subunits described in vertebrates: α1 to α10, β1 to β4, γ, δ, and ε. The α8 subunit is found in the avian genome [3] and is not present in the human genome. The difference between α and β subunits lies within the structure of the ligand-binding site—an α-like subunit is considered to contribute to the ligand-binding site with a principal (+) subunit interface, which includes loops A, B, and C, whereas a β-like subunit is considered a complementary (−), contributing with loops D, E, and F to the ligand-binding interface. The α2, α3, α4, and α6 subunits, through their association with β2 or β4 subunits, can form heteromeric receptors, with different stoichiometries of 2(α)3(β) or 3(α)2(β). More than one type of α and/or β subunits within a receptor could increase receptor diversity. α5 and β3 subunits are considered auxiliary subunits, not contributing to the ACh binding site but influencing properties of the receptor [4,5]. α7 and α9 subunits can assemble into a homomeric receptor. However, association with other subunits is also possible. For example, α7 can associate with β2 [6], while α9 associates with the α10 subunit [7]. α1, β1, δ, ε, and γ are subunits within muscle-type of nAChR, with ε subunits present in adult receptor type, while γ subunit present in fetal receptor.

Each subunit contains an N-terminal extracellular domain (ECD) which contributes to the formation of an orthosteric ligand-binding site, a transmembrane domain containing four α-helices, out of which a second helix contributes to channel pore formation, and an intracellular domain (Figure 1). The orthosteric ligand-binding site is formed at the junction
between two neighboring subunits, where loops A, B, and C, from principal subunits and loops D, E, and F, coalesce and form a hydrophobic cage from aromatic residues that provides a space for ACh binding. The aromatic residues protruding to the ligand-binding site are rather conserved; within the muscle α1 subunit, they include Tyr 93 from loop A, Trp 149 from loop B, and Tyr 190 and Tyr 198 from loop C. These aromatic residues within the principal subunit are assisted by aromatic residues of a complementary subunit, such as Trp 57 of the γ subunit [8]. The conserved aromatic residues interact with ligands via cation-π interaction.

The receptor structure was studied at an atomic level. The quest for receptor structure determination was facilitated through studies on acetylcholine-binding protein (AChBP), a soluble multimeric protein secreted by snail glial cells which buffers ACh concentration [9].

Figure 1. Representation of the structure of nAChR: (A) Cartoon structure of a muscle-like nAChR with pentameric organization shown through side and top views. Ligand-binding extracellular domain, pore-forming transmembrane domain, and an intracellular domain are shown. PDB accession number is 2BG9. (B) Cartoon representation of assembled muscle and neuronal nAChR subtypes. The pentameric organization of the receptor is shown. The triangles represent the subunit interfaces where endogenous ligands and competitive antagonists bind. In the muscle receptor, a γ-subunit is present in fetal form, while an adult receptor contains the ε-subunit. Two ligand-binding sites include α/δ and α/ε or α/γ interfaces, where the α subunit is considered principal, contributing with loops A, B, and C, whereas δ, ε, and γ are complimentary subunits, contributing with loops D, E, and F to the ligand-binding interface.

The receptor structure was studied at an atomic level. The quest for receptor structure determination was facilitated through studies on acetylcholine-binding protein (AChBP), a soluble multimeric protein secreted by snail glial cells which buffers ACh concentration [9].
1.2. Importance of Nicotinic Acetylcholine Receptors in Physiology and Pathology

Acetylcholine is a widely distributed excitatory neurotransmitter. Within the human body, it is present in both branches of the autonomic nervous system: within the parasympathetic system in pre- and postganglionic cells, and within the sympathetic system in preganglionic cells. It is also a neurotransmitter at the periphery within the neuromuscular junction. nAChRs are widely distributed within the central nervous system (CNS) and peripheral nervous system, as well as outside the nervous system. The nucleus basalis of Meynert consists of cholinergic neurons widely projecting into different brain locations [10]. The most prevalent receptor subtype within the brain is the \( \alpha_4\beta_2 \)-containing receptor, the upregulation of which is observed following chronic nicotine consumption [11]. Existing in two stoichiometric forms (\( (\alpha_4)_2(\beta_2)_3 \) and \( (\alpha_4)_3(\beta_2)_2 \)), \( \alpha_4\beta_2 \)-containing receptors can vary in their sensitivities to ACh [12]. nAChRs play an important role in the regulation of neurotransmitter release within central synapses, in activating the muscle at periphery. They play critical role within reward pathways, in particular, within nigrostriatal dopaminergic circuitry [13]. Recent discoveries have expanded the potential role of the receptors, and their significance was shown for pain signal transmission, particularly for \( \alpha_9\alpha_{10} \) nAChR [14–16]. The receptors play a key role in an anti-inflammatory pathway, with an involvement of immune cells and vagal nerve activation [17].

Disfunction in nAChRs within neuromuscular junctions can lead to myasthenic syndrome. Within the CNS, abnormal functioning of the receptors can manifest itself as Alzheimer’s disease, schizophrenia, depression, and Parkinson’s disease [18]. Outside the CNS, improper functionality of the receptors can lead to skin disorders such as pemphigus vulgaris and cancer.

Thus, knowledge about the functionality of the receptors is of paramount importance. Ligands targeting the receptors can help decipher physiological mechanisms, as well as prevent pathological processes where receptors are involved [19,20]. Natural products and, in particular, venoms of venomous animals, such as snails, snakes, spiders, and scorpions, contain biologically active compounds targeting nAChRs; thus, they are actively and thoroughly investigated [21].

2. Snail Venoms as a Source of Toxins Acting on Acetylcholine Receptors

Predatory snails within the Conus genus produce peptides—conotoxins that target different ion channels [22]. Conotoxins differ in their number of disulfide bonds. The peptides with a general structure CC-Xm-C-Xn-C, where C represent cysteines, Xm and Xn—variable number of residues between cysteines—constitute the family of \( \alpha \)-conotoxins (\( \alpha \)-Ctx). \( \alpha \)-Conotoxins are competitive antagonists of nAChRs [23,24]. The different number of residues within two loops contributes to the division of the \( \alpha \)-conotoxins into several subgroups (3/5, 4/3, 4/4, 4/5, 4/6, and 4/7). Structurally, \( \alpha \)-conotoxins have very rigid three-dimensional structure due to the restraints imposed by disulfide bonds. Many \( \alpha \)-conotoxins have a conserved proline in loop 1 between Cys 1–Cys 3, which contributes to structural stability. \( \alpha \)-conotoxins with four residues in loop 1 form a small 3_10 \( \alpha \)-helical region. Functionally, \( \alpha \)-conotoxins could be divided into two groups: those targeting muscle-type nAChRs (\( \alpha 3/5 \) conotoxins) and those targeting neuronal nAChRs (\( \alpha 4/3, \alpha 4/4, \alpha 4/5, \alpha 4/6 \) and \( \alpha 4/7 \) conotoxins).

2.1. \( \alpha 3/5 \) Conotoxins

\( \alpha 3/5 \) conotoxins predominantly target muscle subtypes of nAChRs [25]. The two most well-studied of them include MI and GI conotoxins. \( \alpha \)-Conotoxins MI, GI, and SIA have been shown to exhibit >10,000-fold selectivity for \( \alpha /\delta \) over the \( \alpha /\gamma \) site in mammalian receptors (Table 1). However, the reverse pattern is observed in Torpedo receptors. Conserved Pro 6 and Tyr 12 residues are critical for hydrophobic interaction between the \( \delta \) subunit and MI [26].

Ac1.1a and Ac1.1b are almost identical \( \alpha 3/5 \) conotoxins differing in only one residue at position 14 [27] (Figure 2). They exhibit selectivity towards \( \alpha /\delta \) over \( \alpha /\gamma \) site. The first
three residues before the Cys 4 have been shown to lack an effect on binding the target.

α-Conotoxin GI blocks muscle-type nAChRs, with selectivity for the α/δ site in mouse muscle receptors [28], and the α/γ site in Torpedo californica electric organ receptors [29].

Pro 5, Gly 8, Arg 9, and Tyr 11 are critical residues for GI to target mouse α1β1δε nAChRs. [E1A] GI mutant demonstrated a three-fold increase in potency in mouse α1β1δε and a decreased potency in rat neuronal α9α10 nAChR, compared to wildtype GI [30].

NMR structures for both CIA and CIB alpha-conotoxins derived from Conus catus have been studied. α-Conotoxin CIA caused muscle paralysis in fish when tested in vivo [31], while 4/7 α-Conotoxin CIB blocked neuronal type nAChRs.

Overall, there are two conserved regions (residues 2 and 3 in the first cysteine loop, as well as residues 1, 2, and 4 in the second cysteine loop) inherent to a3/5 conotoxins (Figure 2), which include important residues for the binding to muscle-type nAChR. Notably, SII differs from the rest in two main ways: (1) it has Pro residue in place of expected Arg/Lys; (2) it has a long C-terminus. Structure–functional studies on SII are very scarce, except for one that characterized SII as possessing three disulfide bonds and no net positive charge [32].

2.2. a4/3 Conotoxins

Asp 5, Pro 6, Arg 7, and Trp 10 are critical residues of the ImI conotoxin, conferring its specificity for the α7 nAChRs [33].
Despite the fact that ImI and ImII conotoxins share a high sequence homology (9 of 12 amino acids are identical) (Figure 2), they were found to target different sites at the α7 nAChR, since only ImI (and not ImII) showed competitive inhibition of the receptor when α-bungarotoxin (α-Bgtx) was co-added [34]. Mutational studies revealed that the amino acid residue at position 6 (Pro in α-Ctx ImI, Arg in α-Ctx ImII) determines the selectivity for a specific site, whereas the amino acid residue in position 9 (Ala in α-Ctx ImI, Arg in α-Ctx ImII) confers each of the toxins its optimal affinity to bind to their corresponding sites at the α7 nAChR [34].

The selectivity and potency of α-conotoxin RgIA for the α9α10 subtype of nAChR is conferred by Arg 9 and Arg 13 residues [35]. It is also interesting to note that [3,12]-dicarba RgIA is able to act specifically at α9α10 nAChRs, losing its effect on N-type calcium channels [36]. This analogue could potentially be used in a clinical trial for eliciting an effect on a single receptor subtype.

2.3. α4/4 Conotoxins

α-conotoxin BuIA blocks α6/α3β2 nAChRs with a significantly lower IC50 than α4β2 receptors [37]. In fact, there is a 40,000-fold difference between their IC50 values [37]. The five residues of the α6 subunit, Lys 85, Asp 187, Ile 188, Thr 198, and Tyr 205, were determined to be responsible for this effect [38].

It should be noted that α-conotoxin BuIA on nAChRs with β4 subunits have slow off-time when compared to corresponding nAChRs with β2 subunits [37]. This implies that BuIA can discriminate between different β subunits based on the time needed to unblock the receptor.

The α-conotoxin BuIA [T5A; P6O] was found to be selective for α6β4 vs. α6β2 nAChRs [39]. It was mainly achieved by P6O substitution, which resulted in a 2800-fold increase of IC50 at α6/α3β2β3 and only a 6-fold increase at α6/α3β4 [39].

It is interesting to note that, though α-conotoxins EIIA and PIB come from different Conus species, they are almost identical in sequence, except for one residue at the second position. Therefore, it is not surprising that they both have selectivity for muscle-type nAChRs [40]. This could be due to Asn 4, Pro 5, Ala 6, and Lys 9 residues located in the first and second loops, respectively, which were previously found to be critical for the muscle nAChR selectivity of α3/5 conotoxins [40].

2.4. α4/6 Conotoxins

Alanine scanning mutagenesis revealed three residues in the AuIB conotoxin (Gly 1, Pro 6, and Phe 9) that affect the inhibition of α3β4 nAChRs [41]. Gly 1 participates in the formation of a salt bridge between the N-terminus of the peptide and Asp 72 of the β4 subunit. Hence, its substitution with alanine disrupted a favorable interaction of AuIB with the receptor. However, most of the inhibitory effect is elicited particularly by Pro 6 and Phe 9. It was elucidated that Pro 6 caused inhibition due to its effect on 3D structure of the peptide, whereas Phe 9 was responsible for the interaction with a two-residue binding pocket (Trp 59 and Lys 61) of the β4 subunit [41].

VlIA is a unique α-conotoxin that specifically targets the α3β2 nAChR subtype [42]. Structure–activity studies on the VlIA conotoxin revealed that Arg 1 and His 11 are critical residues for binding to the target effectively. Interestingly, a mutant VlIA[+16L] (a mutant with additional Leu residue at position 16) produced a 12-fold stronger interaction with α3β2 nAChR when compared to the native peptide. This was explained by hydrophobicity Leu residue conferred to the overall structure of the VlIA conotoxin [42].

The VnIB conotoxin selectively inhibits α6β4 nAChRs [43]. However, no structure–activity studies on this conopeptide have been carried out so far. It is hypothesized that all of the members of α4/6 conotoxins, including VnIB, exhibit β4 subunit selectivity due to their common 4/6 substructure. The selectivity of VnIB toward α6-containing nAChRs can potentially be explained by the five residues present in the second disulfide-loop, excluding Pro residue (YTKNPN) [43].
There are five residues (His 5, Pro 6, Val 7, Met 11, and Pro 13) of TxID critical for the inhibition of α3β4 and α6/α3β4 nAChRs [44]. [S9A] TxID mutant was found to discriminate between the two receptor subtypes, having a 46-fold higher affinity to α3β4 than to α6/α3β4 nAChRs [44].

2.5. α4/7 Conotoxins

Ala 11 and Gln 14 play a role in α3β2 selectivity of AnlB, whereas the C-terminal amidation and sulfation of Tyr 16 are responsible for α7 nAChR binding [45].

ArIB [V11L; V16D] is considered to be the most selective α7 nAChR antagonist to date (IC50 = 1.09 nM) [46].

Important residues conferring EI potency to bind mouse α1β1δε nAChR were found to be His 7, Pro 8, Met 12, and Pro 15. In addition, deletion of Arg1–Asn2–Hyp3 residues are also crucial, since their deletion causes a total loss of activity at muscle receptors [47]. [S13A] EI mutant exhibited increased potency and selectivity for α1β1δε nAChRs [47].

GIC is a very interesting conotoxin from a research standpoint, since it exhibits extreme potency and selectivity towards α3β2 nAChRs (IC50 = 1.1 nM) [48]. His 5 and Gln 13 were found to be the most important residues that confer GIC its potency and selectivity, respectively. In addition, Ala 7, Asn 11, and Asn 12 residues of the peptide were involved in binding to the principal site of the receptor [48].

Ala 10, Val 13 and Val 18 are important residues of the GID conotoxin, conferring its selectivity for α4β2 over α3β2 nAChRs [49]. In particular, GID[V18N] analog exhibits the highest selectivity for α4β2, with no inhibitory effect on the α3β2 subtype [49].

The structure–functional study conducted to the Lo1a conotoxin found that its Asp-18 residue is critical for the selectivity of the peptide for neuronal vs. muscle subtype nAChRs [50]. This was evident from the observation that Lo1a-AD and Lo1a-RRR analogues, which either lacked Asp 18 or had it replaced with 3 Arg residues, respectively, acquired affinity for the adult muscle subtype α1β1δε, aside from α7 receptors [50].

Ser-1 and Gly 2 are important residues for the affinity of LsIA to α3β2 and α7 nAChRs [51]. In addition, carboxylation of the C-terminus of LsIA resulted in its selectivity for α3β2 over α7 to increase 9-fold [51].

Selectivity of the Lt1.3 conotoxin for α3β2 receptors is attributed to a small hydrophobic Gly 10 residue, whereas Asn 11, Asn 12, Pro 13, Tyr 14, and Phe 15 residues contribute to the receptor binding [52].

His 5, Pro 6, Ala 7, and His 12 of LsIA are important residues that interact with α3-containing nAChRs [53]. LsIA exhibits unusual selectivity toward α3β2 vs. α6/α3β2β3 nAChRs due to Asn 9 and Asp 11 residues [54].

Asp 5, Pro 6, and His 12 are the main residues of the MII conotoxin, which confers its potency for binding to the rat α3β2 nAChR subtype [55]. The MII [H9A; L15A] analog of α-conotoxin MII is selective towards α6-containing receptors (IC50 on rat α6/α3β2β3 = 2.4 nM), and can discriminate between α6 and α3 subunits [56].

Mr1.7 [E2A] is potent and selective for the α3β2 nAChR subtype, with no inhibitory effect on other subtypes [57]. However, the substitution of Ser 12 with Ala results in the loss of selectivity to α3β2, with new binding ability to other subtypes (α3β4, α2β4, and α7) [57].

The PeIA conotoxin can be made >15,000-fold more potent on α6/α3β2β3 vs. α3β2 nAChRs via substituting Asn 11 with either Lys or Arg [58]. On the contrary, PeIA-4566, which is a synthetic peptide composed of non-natural amino acids, targets α3β2 over α6/α3β2β3, with a 300-fold difference in potency [59]. PeIA [S9H; V10A; E14N] analog blocks α6/α3β2β3 and α6/α3β4 nicotinic receptors with IC50 values of 223 pM and 65 nM, respectively [60]. Thus, it can be concluded that PeIA [S9H; V10A; E14N] discriminates between α6β2 and α6β4 nAChRs.

Residues from Leu 5 to Pro 13 and Tyr 15 are important residues of PnIA, conferring its inhibitory effect on α7 nAChRs [61]. In addition, the PnIA [A10L] mutant is able to exclusively bind α7 nAChRs [62].
The RegIIA [N11A; N12A] analog selectively blocks \( \alpha_3\beta_4 \) vs. \( \alpha_3\beta_2 \) nAChRs, with a 1000-fold difference in potency [63].

TxB is unique due to its ability to selectively bind \( \alpha_6/\alpha_3\beta_3 \) nAChRs, while lacking any capability to bind to other receptor subtypes [64]. However, structure–functional studies accounting for this have not been conducted yet.

It is generally accepted that \( \alpha \)-Ctxs are antagonists of nAChRs, but MrIC (PECC-THPACHVSNPELC), which is a peptide variant of Mr1.7, plays a full agonist role of endogenous human \( \alpha_7 \) nAChRs in the presence of PNU (positive allosteric modulator of \( \alpha_7 \) nAChR subtype) [65]. It is suggested that the Pro residue in the N-terminus of the peptide has a role in binding \( \alpha_7 \) nAChR [65].

It was found that Asp 5 to Arg 7, and Asp 11 to Ile 15, are critical residues for the inhibitory effect of Vc1.1 at the \( \alpha_9\alpha_10 \) nAChR subtype [66]. In addition, mutations at positions 4 and 9 (substitution of Ser 4 with either Lys or Arg and substitution of Asn 9 with either Ala, Leu, or Ile) increased the potency of Vc1.1 at \( \alpha_9\alpha_10 \) nAChRs [66].

### 2.6. Therapeutic Potential of \( \alpha \)-Conotoxins

Due to the fact that nAChRs are implicated in a range of pathological conditions, \( \alpha \)-conotoxins targeting nAChRs pose themselves as attractive candidates for drug development [67–69]. For example, \( \alpha \)-conotoxins RgIA and Vc1.1 demonstrate the analgesic effect in animal models of neuropathic pain [14,70–76]. Such analgesic effect works through targeting \( \alpha_9\alpha_10 \) nAChRs. The mechanism of analgesia mediated by RgIA and Vc1.1 conotoxins is not currently clear, but there are indications that it involves immune-mediated pathways.

RgIA was shown to be potent in reducing mechanical allodynia and oxaliplatin-induced hypersensitivity to thermal and mechanical stimuli [77], as well as accelerating the recovery of nerve damage following chronic constriction injury (CCI) [70]. Other than alleviating neuropathic pain, RgIA was also shown to be able to reduce the severity of dextran sodium sulfate-induced colitis in an animal model [78].

Vc1.1 was shown to reduce mechanical allodynia in rats with diabetic neuropathy induced by streptozotocin, and in rats with CCI [14,74]. Vc1.1 reduced mechanical hyperalgesia in rats with peripheral nerve ligation when the toxin was injected intramuscularly [75].

Another \( \alpha \)-conotoxin with a potential therapeutic application due to its antitumor activity is TxD. The toxin inhibited the growth of lung cancer cells [79].

The results from in vitro and animal studies serve as an encouragement for drug development. There are, however, hindrances along the way [80], which stem from the peptidic nature of the toxins, the potential off-target effects, and different potencies on human receptors vs. rodent ones. For example, Vc1.1 was discontinued from phase II human clinical trials due to its low affinity on human \( \alpha_9\alpha_10 \) nAChRs [15] (\( IC_{50} \) on rat \( \alpha_9\alpha_10 \) is 20–100 nM, on human \( \alpha_9\alpha_10 \) is 1 \( \mu \)M).

### Table 1. The \( \alpha \)-conotoxins mentioned in the main text are shown, together with their targets and potencies.

| \( \alpha \)-Conotoxin | Species | Target and \( IC_{50} / Ki \) | Reference | UniProt/PDB |
|-----------------------|---------|-----------------------------|-----------|-------------|
| Ac1.1a                | C. achatinus | m(\( \alpha_1 \))2\( \beta_1\)1\( \delta\)1\( \gamma \) (\( IC_{50} = 36 \) nM) | [27] | P0CAQ4/n.d. |
| Ac1.1b                | C. achatinus | m(\( \alpha_1 \))2\( \beta_1\)1\( \delta\)1\( \gamma \) (\( IC_{50} = 26 \) nM) | [27] | P0CAQ5/n.d. |
| CIA                   | C. catus | r(\( \alpha_1 \))2\( \beta_1\)1\( \delta\)1\( \gamma \) (\( IC_{50} = 5.7 \) nM) r\( \alpha_3\beta_2 \) (\( IC_{50} = 2.06 \) \( \mu \)M) | [31] | D4HPD6/n.d. |
| α-Conotoxin | Species       | Target and IC$_{50}$/Ki                                                                 | Reference | UniProt/PDB |
|------------|---------------|-----------------------------------------------------------------------------------------|-----------|-------------|
| GI         | C. geographus | Torpedo californica (IC$_{50}$ α/γ site = 4.5 nM)                                       | [29,30]   | P01519/1XGA |
|            |               | m(α1)β1γ                                                (IC$_{50}$ α/δ site = 1.3 nM)               |           |             |
|            |               | m(α1)β1γ                                                (IC$_{50}$ α/γ site = 60 µM)                 |           |             |
|            |               | ra9α10 (IC$_{50}$ = 9.35 µM)                           |           |             |
| MI         | C. magus      | m(α1)β1γ                                                (IC$_{50}$ = 4.0 nM)                        | [26]      | P01521/n.d. |
| SIA        | C. striatus   | m(α1)β1γ                                                (IC$_{50}$ = 2.6 nM)                        | [28]      | P28878/n.d. |
| SII        | C. striatus   | m(α1)β1γ                                                (IC$_{50}$ = 2.3 µM)                        | [28]      | P28879/6OTB |
| ImI        | C. imperialis | ha3β2 (IC$_{50}$ = 40.8 nM)                           | [34,81]   | P50983/1CNL |
|            |               | ha3β4 (IC$_{50}$ = 3.39 µM)                            |           |             |
|            |               | ha7 (IC$_{50}$ = 596 nM)                               |           |             |
|            |               | ra7 (IC$_{50}$ = 191 nM)                               |           |             |
| ImII       | C. imperialis | h(α1)β1γ                                               (IC$_{50}$ = 1.06 µM)                     | [34,81]   | Q8I6R5/n.d. |
|            |               | ha3β2 (IC$_{50}$ = 9.61 µM)                            |           |             |
|            |               | ha7 (IC$_{50}$ = 571 nM)                               |           |             |
|            |               | ra7 (IC$_{50}$ = 441 nM)                               |           |             |
| RgIA       | C. regius     | ra9α10 (IC$_{50}$ = 4.55–5.19 nM)                      | [82]      | P0C1D0/2JUT |
|            |               | ra7 (IC$_{50}$ = 4.66 µM)                              |           |             |
| BuIA       | C. bullatus   | ra6/α3β2 (IC$_{50}$ = 0.3 nM)                          | [37,39]   | P69657/2I28 |
|            |               | ra6/α3β2β3 (IC$_{50}$ = 0.46 nM)                        |           |             |
|            |               | ra6/α3β4 (IC$_{50}$ = 1.5–2.1 nM)                       |           |             |
|            |               | ra2β4 (IC$_{50}$ = 121 nM)                             |           |             |
|            |               | ra3β2 (IC$_{50}$ = 9.57 nM)                            |           |             |
|            |               | ra3β4 (IC$_{50}$ = 28 nM)                              |           |             |
|            |               | ra4β4 (IC$_{50}$ = 70 nM)                              |           |             |
|            |               | ra7 (IC$_{50}$ = 272 nM)                               |           |             |
| EIIA       | C. ermineus   | Torpedo (Ki = 0.46–105 nM)                            | [40]      | D4HRK4/n.d. |
|            |               | α7-5HT3 (Ki >>1000 nM)                                 |           |             |
|            |               | α3β2 (Ki >>1000 nM)                                   |           |             |
| PIB        | C. purpurascens | m(α1)β1γ                                               (IC$_{50}$ = 36 nM)                     | [83]      | P0C351/n.d. |
|            |               | m(α1)β1γ                                               (IC$_{50}$ = 45 nM)                     |           |             |
| AuIB       | C. aulicus    | α3β2/α3β4 (intracardiac ganglia)                       | [84,85]   | P56640/1MXN |
|            |               | (IC$_{50}$ = 1.2 nM)                                   |           |             |
|            |               | ra3β4 (recombinant)                                   |           |             |
|            |               | (IC$_{50}$ = 750–966 nM)                              |           |             |
| ViIA       | C. virgo      | ra3β2 (IC$_{50}$ = 845.5 nM)                          | [42]      | F5C0A0/n.d. |
| VnIB       | C. ventricosus| ra6β4 (IC$_{50}$ = 12 nM)                             | [43]      | A0A4P8XV20/n.d. |
|            |               | ra6/α3β4 (IC$_{50}$ = 18 nM)                          |           |             |
|            |               | ra3β4 (IC$_{50}$ = 320 nM)                            |           |             |
|            |               | ra6/α3β2β3 (IC$_{50}$ = 4000 nM)                       |           |             |
|            |               | ha6/α3β4 (IC$_{50}$ = 5.3 nM)                          |           |             |
| TxDI       | C. textile    | ra3β4 (IC$_{50}$ = 12.5 nM)                           | [64]      | K8DWB5/n.d. |
|            |               | ra6/α3β4 (IC$_{50}$ = 94 nM)                          |           |             |
| AnIB       | C. anemone    | ra3β2 (IC$_{50}$ = 0.3 nM)                            | [45]      | P0C1V7/n.d. |
Table 1. Cont.

| α-Conotoxin | Species       | Target and IC \textsubscript{50} / Ki                                      | Reference        | UniProt/PDB          |
|-------------|---------------|-------------------------------------------------------------------------|------------------|----------------------|
| ArIA        | C. arenatus   | ra7 (IC\textsubscript{50} = 6 nM)                                      | [46]             | P0C8R2/n.d.          |
|             |               | ra3β2 (IC\textsubscript{50} = 18 nM)                                   |                  |                      |
| ArIB        | C. arenatus   | ra7 (IC\textsubscript{50} = 1.8 nM)                                    | [46]             | P0C8R2/n.d.          |
|             |               | ra6/α3β2β3 (IC\textsubscript{50} = 6.45 nM)                            |                  |                      |
|             |               | ra3β2 (IC\textsubscript{50} = 60.1 nM)                                 |                  |                      |
| CIB         | C. catus      | ra3β2 (IC\textsubscript{50} = 128.9 nM)                                | [24]             | P0DPT2/n.d.          |
|             |               | ra7 (IC\textsubscript{50} = 1.51 µM)                                   |                  |                      |
| El          | C. ermineus   | m(α1)2β1δε (IC\textsubscript{50}(1) = 9.4 nM)                           | [86]             | P50982/1K64          |
|             |               | (IC\textsubscript{50}(2) = 280 nM)                                     |                  |                      |
|             |               | Torpedo (IC\textsubscript{50}(1) = 0.41 nM)                            |                  |                      |
|             |               | (IC\textsubscript{50}(2) = 190 nM)                                     |                  |                      |
| GIC         | C. geographus | ha3β2 (IC\textsubscript{50} = 1.1 nM)                                  | [87]             | Q86RB2/1UL2          |
|             |               | ha3β4 (IC\textsubscript{50} = 755 nM)                                  |                  |                      |
|             |               | haαβ2 (IC\textsubscript{50} = 309 nM)                                  |                  |                      |
| GID         | C. geographus | ra3β2 (IC\textsubscript{50} = 3.1 nM)                                  | [88,89]          | P60274/1MTQ          |
|             |               | ra7 (IC\textsubscript{50} = 4.5 nM)                                    |                  |                      |
|             |               | ra4β2 (IC\textsubscript{50} = 152 nM)                                  |                  |                      |
| Lo1a        | C. longurionis| α7 (IC\textsubscript{50} = 3.24 µM)                                   | [50]             | X1WB75/2MD6          |
| LsIA        | C. limpusi    | α3β2 (IC\textsubscript{50} = 10 nM)                                    | [51]             | P0DL68/n.d.          |
|             |               | α3α5β2 (IC\textsubscript{50} = 31 nM)                                  |                  |                      |
|             |               | α7 (IC\textsubscript{50} = 10 nM)                                      |                  |                      |
| LtIA        | C. litteratus | α3β2 (IC\textsubscript{50} = 9.8 nM)                                   | [90]             | Q2I2R8/n.d.          |
|             |               | α6/α3β2β3 (IC\textsubscript{50} = 84.4 nM)                             |                  |                      |
|             |               | α6/α3β4 (IC\textsubscript{50} = 6 µM)                                  |                  |                      |
| Lt1.3       | C. litteratus | ra3β2 (IC\textsubscript{50} = 44.8 nM)                                 | [52]             | n.d.                 |
| LvIA        | C. lividus    | α3β2 (IC\textsubscript{50} = 8.7 nM)                                   | [53]             | L8BU87/2MDQ          |
|             |               | α6/α3β2β3 (IC\textsubscript{50} = 108 nM)                              |                  |                      |
|             |               | α6/α3β4 (IC\textsubscript{50} = 121 nM)                                |                  |                      |
|             |               | α7 (IC\textsubscript{50} = 3 µM)                                       |                  |                      |
|             |               | ha3β2 (IC\textsubscript{50} = 17.5 nM)                                 |                  |                      |
|             |               | haαβ2 (IC\textsubscript{50} = 5.34 µM)                                 |                  |                      |
| MII         | C. magus      | α6/α3β2β3 (IC\textsubscript{50} = 0.4 nM)                              | [55,56,91]       | P56636/1M2C          |
|             |               | αβ2 (IC\textsubscript{50} = 0.5/3.7 nM)                                |                  |                      |
| Mr1.7       | C. marmoreus  | ra3β2 (IC\textsubscript{50} = 53.1 nM)                                 | [57]             | F6LPN3/n.d.          |
|             |               | ra9α10 (IC\textsubscript{50} = 185.7 nM)                               |                  |                      |
|             |               | ra6/α3β2β3 (IC\textsubscript{50} = 284.2 nM)                            |                  |                      |
| PeIA        | C. pergrandis | ra9/α10 (IC\textsubscript{50} = 6.9 nM)                                | [92]             | Q1L777/n.d.          |
|             |               | ra7 (IC\textsubscript{50} = 1.8 µM)                                    |                  |                      |
|             |               | ra3β2 (IC\textsubscript{50} = 23 nM)                                   |                  |                      |
|             |               | ra3β4 (IC\textsubscript{50} = 480 nM)                                  |                  |                      |
| PnIA        | C. pennaceus  | ra3β2 (IC\textsubscript{50} = 9.56 nM)                                 | [61]             | P50984/1PEN          |
|             |               | ra7 (IC\textsubscript{50} = 252 nM)                                    |                  |                      |
| RegIIA      | C. regius     | ra3β4 (IC\textsubscript{50} = 97 nM)                                   | [93]             | P85013/n.d.          |
|             |               | ra3β2 (IC\textsubscript{50} = 33 nM)                                   |                  |                      |
|             |               | ha7 (IC\textsubscript{50} = 103 nM)                                   |                  |                      |
Table 1. Cont.

| α-Conotoxin | Species | Target and IC\textsubscript{50} / Ki | Reference | UniProt/PDB |
|-------------|---------|-------------------------------------|-----------|------------|
| TxIB        | C. textile | rα6/α3β2β3 (IC\textsubscript{50} = 28 nM) | [64] | K4RNX9/2LZ5 |
| Vc1.1       | C. victoriae | rα9α10 (IC\textsubscript{50} = 109 nM), hxα9α10 (IC\textsubscript{50} = 549 nM), rα6/α3β2β3 (IC\textsubscript{50} = 140 nM), rα3β4 (IC\textsubscript{50} = 4.2 µM), rα3β2 (IC\textsubscript{50} = 7.3 µM) | [66,94] | P69747/2H8S |

3. Snake Venoms as a Source of Toxins Acting on Acetylcholine Receptors

Snakes are a rich source of ligands targeting nAChRs. The neurotoxins of snake origin were characterized long ago and include α-Bgtx from Bungarus melliteucus and α-cobratoxin (α-Cbtx) from Naja naja siamensis. The biologically active toxins in snake venoms can be organized into molecules with enzymatic or without enzymatic activities. Many snake neurotoxins of non-enzymatic action possess a conserved structural feature consisting of three-finger loops, where β-sheet fingers protrude from a globular hydrophobic core coordinated by disulfide bonds [95]. Neurotoxins targeting postsynaptic nAChRs are called α-neurotoxins (α-NTX) [96]. Depending on the length and number of disulfide bridges, three-finger α-neurotoxins are divided into short-chain toxins (type I) and long-chain toxins (type II). Long-chain toxins contain 66–75 amino-acids and ten cysteines, whereas short-chain toxins have 60–62 amino-acids and eight cysteines. Other than three-fingered neurotoxins with a typical structure, more and more toxins with alternate structures are being discovered. For example, non-conventional three-finger neurotoxins are similar to long-chain α-NTX in their length and number of disulfide bonds; however, the fifth disulfide bond is located within finger I of the toxin, unlike in finger II within long-chain α-NTX [97]. The toxins within this group include “weak toxin” WTX [98] and candoxin [99]. Some snake toxins can form homo- or heterodimers. Homodimers of short-chain three-finger α-NTX include fulditoxin and haditoxin. Homodimers of long-chain α-NTX include the α-cobratoxin dimer [100,101]. Homodimers can be formed as a result of covalent interactions, as in α-cobratoxin, or noncovalent interactions, as in κ-bungarotoxin, fulditoxin, and haditoxin [101,102]. Heterodimeric toxins include irditoxin with intermolecular disulfide bonds [103].

3.1. Long-Chain Three-Finger α-NTxs

Long-chain three-finger α-NTs interact with muscle and neuronal nAChRs. To the neuronal α7 receptor, these toxins bind with high affinity, K\textsubscript{d} values at 10\textsuperscript{−9} to 10\textsuperscript{−8} M [104]. The residues within finger II of the toxin were shown to be important for binding to this receptor type. The mode of interaction of two long-chain three-finger α-NTxs, α-bungarotoxin and α-cobratoxin, will be considered below.

α-bungarotoxin contains 74 amino-acids and 5 disulfide bonds. The toxin was isolated in 1963 by Chang and Lee [105] from a Taiwan banded krait Bungarus multicinctus, and helped in the characterization of muscle-type nAChRs [106]. Original discovery of the toxin demonstrated its paralytic effect in rodent muscle preparation, and later it was shown that the toxin targets muscle nAChRs with high affinity (Kd ~ 5 nM), acting as a competitive antagonist in an irreversible fashion [104]. The toxin also blocks Torpedo acetylcholine receptor in the irreversible fashion [107]. Besides targeting muscle-type receptor, it also has high affinity towards homomeric neuronal receptors, such as α7 and α9, with nanomolar affinity [108]. An NMR structure of the toxin (PDB: 1KFH) shows three fingers projecting from the globular core, shaped by four disulfide bonds, with an additional disulfide bond within finger II. Structure–functional studies revealed significance of cation–π interaction in stabilizing the toxin within the ligand-binding site. Arg 36 plays a major role in toxin binding to muscle receptor type, as well as for neuronal receptor type [109]. This is
due to similarities in the structure of guanidinium group within Arg 36 and quaternary ammonium of acetylcholine, which directs toxins into the agonist binding site.

Multiple crystal structures of the toxin bound to its target were solved, which helped in understanding of the mode of toxin–ligand interaction by revealing the orientation of the toxin within binding site of the receptor. Moreover, 1.94 Å crystal structure of α-Bgtx bound to extracellular domain of α1 subunit of mouse muscle receptor is available (PDB: 2QC1) [110], which reveals the N-glycosylated Asn 141 on the receptor side, with oligosaccharide composed of two N-acetylglucosamines (GlcNAc 2–3) and eight mannoses (Man 4–11), and forming hydrogen bonds with the toxin. The study demonstrated the orientation of the toxin within an aromatic cage of the ligand-binding interface. Finger I is surrounded by loop C and carbohydrate; the tip of finger II of the toxin is oriented into the ligand-binding site made up by loops A, B, and C on the receptor side, and finger III is away from the receptor. Arg 36 and Phe 32 within finger II on the toxin side form a cation-π stack and oppose Tyr 198 from the receptor side in a “face-to-face” fashion. Two other tyrosines on the receptor side, Tyr 190 and Tyr 93, are also oriented towards Arg 36 and Phe 32, but face their edges. The structure also revealed hydrogen bonds between Arg 36, Thr 148, and Cys 192. Despite the wealth of information obtained from this crystal structure, the lack of adjacent subunit obscures the entire picture. Additionally, Trp 149 to Arg substitution, which was performed for crystallization purposes, removed Trp, which is important for toxin–receptor interaction.

The crystal structure of the α-Bgtx bound to human α7–AChBP chimera resolved to 3.5 Å provided insight into the toxin interaction with adjacent subunits within a ligand-binding interface (PDB: 4HQP), where loop C of the receptor made contact with finger II of the toxin [111]. Particularly, Tyr 184, which is equivalent to Tyr 190 in mouse α1 subunit, is oriented towards the edge of cation-π stack, formed by Arg 36 and Phe 32. This is analogous to the earlier crystal structure. Tyr 184 is itself hydrogen-bonded through hydroxyl-group, with Asp 30 of the α-Bgtx. Other residues from loop A (Tyr 91), loop B (Trp 145), and loop C (Tyr 191, Arg 182) also contribute to the toxin binding.

The crystal structure of α-Bgtx complexed with the extracellular domain of α9 subunit at 2.7 Å was solved (PDB: 4UY2), and it revealed the mode of interaction between the toxin and a principal subunit of the receptor. Thus, Arg 36 and Phe 32 of finger II of the α-Bgtx are positioned deeply in the ligand-binding site of the α9 subunit, where they interact with residues of loops A, B, and C. Particularly, Arg 36 makes a cation-π interaction and hydrogen bond with Tyr 95. Asp 30 forms a hydrogen bond with Tyr 192. Finger I interacts with loop C, with Val 40 forming a hydrogen bond with Ser 191. Finger III is oriented away from the ligand-binding site. Due to the fact that only a principle subunit interface is revealed by the structure, the contribution of the complementary subunit is not seen [112].

The Cryo-EM structure of α-Bgtx complexed with native receptor from Torpedo electric organ (PDB: 6UWZ) revealed details of the toxin–receptor interaction [113] with 2.7 Å resolution. The structure revealed residues on α-δ and α-γ interfaces important for binding. The study highlighted the involvement of residues within complementary side on the ligand-binding interface, particularly within loop F. The structure showed the penetration of finger II of the toxin deep into the ACh binding pocket, where it makes contact with Tyr 93, Tyr 190, and Tyr 198 of the α subunit. Similar to the previous studies, Arg 36 forms a cation-π interaction with Tyr 198 of the principal receptor subunit and Phe 32 of the toxin.

α-cobratoxin is a 71 amino-acid-long toxin from the Indo-Chinese spitting cobra Naja naja siamensis which demonstrates curarimimetic activity. Residues important for binding to muscle-type receptor include Lys 23, Trp 25, Asp 27, Phe 29, Arg 33, and Lys 49. In addition, mutational studies revealed the importance of Arg 36 and Phe 65 for binding to α7 nAChRs and the Torpedo receptor [114], with some residues specific for binding to the α7 receptor ( Ala 28, Lys 35, Cys 26, and 30), while others were specific for binding to the Torpedo receptor (Lys 23, Lys 49). The double-mutant cycle analysis for α-Cbtx interaction with the α receptor revealed Phe 29–Trp 54 coupling [115]. The crystal structure of α-
cobratoxin complexed with AChBP from *Lymnaea stagnalis* [116] revealed more details of a toxin–receptor interaction, where the toxin inserts its finger II deep into the ligand-binding pocket, with Phe29 and Arg33 of the toxin mimicking acetylcholine at the interface between two subunits. Phe29 and Arg33 engage in hydrophobic and aromatic interactions with AChBP Trp53, Tyr185, Tyr192, and Trp143. Additionally, Trp25, Asp27, Ala28, and Ile32 are close to Tyr185 in the principle subunit interface of AChBP and Glu163, Glu55, Leu112, Met114, and Tyr164 in a neighboring complementary subunit. Possible interactions could involve Ser31, Cys26, and Cys30 within finger II of the toxin, and AChBP Ser159–Tyr164 in a complementary subunit on the receptor side. The toxin displaces loop C of the subunit.

αδ-bungarotoxin-1 is a 73 amino-acid-long toxin isolated from *Bungarus candidus*, with five disulfide bonds and inhibitory activity in muscle and neuronal α7 nAChRs. Unlike α-Bgtx, αδ-Bgtx binds to the target receptor in a reversible manner. Binding affinities are higher at the α-δ interface than they are at the α-γ or α-ε interfaces [117]. The reason for such selectivity could be due to the shorter finger I, and thus a smaller number of contacts with the receptor interface, as well as fewer positively charged amino acids within finger II of the toxin.

Drysdalin is a long-chain NTX with 87 amino acids. It has five disulfide bonds and possesses curarimimetic activity with the irreversible inhibition of muscle receptors. The toxin demonstrates nanomolar affinity towards rodent muscle (α1)2β1δε, as well as human α7 and α9α10 nAChRs. Arg 30 and a 24-residues-long C-terminal tail were shown to be important for toxin binding. The toxin has a 24-long C-terminus tail, the truncation of which abolishes the activity of the toxin on α9α10 nAChRs [118]. The toxin has a highly divergent structure within the second finger, where three of eight highly conserved in long-chain NTX residues, important for receptor interaction, are altered. Despite the lack of conserved residues within the second finger, the toxin retains properties of the long-chain three-finger α-NTXs, and causes irreversible postsynaptic neurotoxicity at a nanomolar concentration (IC50 on chick biventer cervices muscle preparation is 38.7 nM). Despite the sequence difference, the toxin retains the target specificity of the long-chain α-NTXs, suggesting functional rather than structural similarity between the long-chain toxins.

3.2. Short-Chain Three-Finger α-Neurotoxins

Erabutoxin a and b are 62 amino-acid-long short-chain neurotoxins isolated from the Chinese sea snake *Laticauda semifasciata* with four disulfide bonds. The toxins are potent inhibitors of the muscle-type receptor, *Torpedo* receptor, and is a typical curarimetic toxin [119]. The toxin is a weak antagonist of homomeric neuronal α7 nAChRs. Mutagenesis studies revealed a contribution of the conserved core residues within finger II, such as Lys 27, Trp 29, Asp 31, Phe 32, and Arg 33 for receptor binding [120,121]. In addition, His 6, Gln 7, Ser 8, and Gln 10 of finger I, as well as additional residues of finger II, such as Tyr 25, Gly 34, Ile 36, and Clu 38, are important for receptor binding.

Nmml is a 62 amino-acid long short-chain neurotoxin isolated from *Naja mosambica* possessing high affinity towards muscle-type nAChR. The neurotoxin is capable of distinguishing α-γ and α-δ subunit interfaces from an α-ε interface; it has an affinity 3 orders of magnitude higher towards the former compared to the latter [122,123]. Mutational studies revealed the significance of Lys 27 for toxin binding, and double-mutant cycle analysis revealed the interaction between Lys 27 and Glu 176 on γ subunit [123].

3.3. Dimeric Toxins

Dimeric toxins demonstrate changes in target specificity compared to a monomeric form, with many dimeric toxins targeting neuronal types of nAChRs. Fulditoxin, haditoxin, dimeric α-cobratoxin, κ-bungarotoxin, and irditoxin are examples of dimeric toxins. Fulditoxin is a homodimer of two short-chain three finger α-NT, with monomers linked together through 29 hydrophobic interactions between residues within Finger II of the monomer [102]. Haditoxin is a homodimer of two short-chain α-3FNTX, with monomers bound to each other through 14 hydrogen bonds between finger III residues [124]. α-
cobratoxin dimer contains two covalently joined monomers bound by two disulfide bonds [100,101]. K-bungarotoxin is a dimeric NTX, where two identical monomers are bound through 9 hydrogen bonds [100]. The dimeric nature of the toxin changes its binding properties, making the toxin lose its potency on muscle receptors while enabling its interaction with neuronal types of receptors (α3β2, α7, and α4β2 nAChRs). Iridotoxin is a heterodimeric toxin with two protomers covalently bound by a disulfide bond. The toxin demonstrates specific activity on avian muscle receptors, with a 1000-times higher activity on avian receptors compared to rodent receptors (10 nM vs. >10 μM IC50) [103] (Table 2).

Table 2. Toxins of snake origin targeting nAChRs.

| Toxin Same (UniProt) | Snake Species | Target (IC50 or Kd Values) | Ref. |
|----------------------|---------------|----------------------------|------|
| α-bungarotoxin (P60615) | Multibanded krait Bungarus multicinctus (Elapidae) | Torpedo nAChRs (IC50 = 0.4 nM) α7 (IC50 = 0.4 nM) α7-SHT3 chimera (IC50 = 0.95 nM) | [119] |
| α-cobratoxin (P31391) | Indo-Chinese spitting cobra Naja naja siamensis (Elapidae) | Ac-ACHBP (IC50 = 191 nM) Ls-AChBP (IC50 = 3.2 nM) α7 (IC50 = 0.5 nM) α7-SHT3 chimera (IC50 = 9 nM) | [119,125] |
| αδ-bungarotoxin-1 | Blue krait Bungarus candidus (Elapidae) | T. californica nAChRs (IC50 = 11.4 nM) α4-β2 (IC50 = 15.7 nM) α1β1β2γ (IC50 = 2.54 nM) | [117] |
| Drysdalin (F8I2B3) | White-lipped snake Drysdalia coronoides (Elapidae) | rodent α1β1β2 (IC50 = 16.9 nM) α7 (IC50 = 10 nM) α9α10 (IC50 = 11.3 nM) | [118,126] |
| Erabutoxin-a (P60775) | Chinese sea snake Laticauda semifasciata (Elapidae) | Torpedo nAChR (Kd = 0.07 nM) α7 (IC50 = 0.5 μM) α7-SHT3 chimera: Erabutoxin a (IC50 = 21 nM) Erabutoxin b (IC50 = 22 nM) | [120,121,122] |
| Erabutoxin-b (Q9OVW1) | | | |
| NmmI | Mozambique spitting cobra Naja mossambica (Elapidae) | α1-γ and α1-δ interfaces (Kd = 100 pM) α1-ε interface (Kd = 100 nM) | [122,123] |
| k-Bungarotoxin homodimer (P01390) | Multibanded krait Bungarus multicinctus (Elapidae) | α3β2 (IC50 = 3 nM) α7 and α4β2 weak inhibition | [128,129] |
| α-cobratoxin homodimer | Monocled cobra Naja kaouthia (Elapidae) | α1β1γ of Torpedo (IC50 = 10 nM) α7 (IC50 = 0.2 μM) α3β2 (IC50 = 0.15 μM) | [100,101] |
| Haditoxin (A8N286) | King cobra Ophiophagus hannah (Elapidae) | α7 (IC50 = 0.2 μM) α1β1γ (IC50 = 0.5 μM) α3β2 (IC50 = 0.5 μM) α4β2 (IC50 = 2.6 μM) | [124] |
| Fulditoxin | Eastern coral snake Micruroides fulvius fulvius (Elapidae) | rxβ1β2 (IC50 = 2.6 μM) rxβ1β2 (IC50 = 1.8 μM) α7 (IC50 = 7 μM) αx3β2 (IC50 = 12.6 μM) reversible blockade | [102] |
| Iridotoxin A and B (A15664, A15665) | Brown tree snake Boiga irregularis (Colubridae) | Species specific activity: avian α1β1γ (IC50 = 10 nM) rodent α1β1γ (IC50 > 10 μM) | [103] |
| Canditoxin (P81783) | Malay krait Bungarus candidus (Elapidae) | αx1β1γ nAChRs (IC50 = 10 nM), reversible block | [97,99] |
| Weak toxin (WTX) (P82935) | Monocled cobra Naja kaouthia (Elapidae) | Torpedo nAChR (Kd = 90 nM) (native toxin) α7 (IC50 = 14.8 μM) (recombinant) T. californica (IC50 = 3 μM) (recombinant) | [98,130,131] |
| Dimnutoxin (Q06ZW0) | Mangrove snake Boiga dendrophila (Colubridae) | Bird specific postsynaptic activity—irreversible inhibition at chick biventer neuromuscular preparation at 10 μg/mL (IC50 = 300 nM) | [132] |
3.4. Non-Conventional Toxins

Weak toxin (WTX) isolated from cobra Naja kaouthia is a 65 amino-acid-long toxin with five disulfide bonds. By length, it is similar to short-chain toxins; by number of disulfide bonds, it is similar to long-chain toxins. However, unlike in long-chain neurotoxins, the fifth bond is within the first finger. The toxin is named “weak” because of its low toxicity. It was reported that, at 2 mg/kg delivered intravenously, it was not toxic on rats [98]. The toxin inhibits Torpedo nAChR with a Kd of 90 nM, and it also shows affinity towards neuronal α7 and muscarinic receptors present within adrenal medulla cells [130]. Arg 31 and Arg 32, both within the second finger, are important for the interaction with muscle and α7 nAChRs [131]. The same residues, together with Arg 37, are crucial for binding to human muscarinic M1 and M3 receptors [139].

Candoxin is a 66-amino-acid-long toxin isolated from Bungarus candidus snake, similar in length to short-chain toxins. However, unlike short-chain toxins, it has five disulfide bonds. The location of the fifth bond is within finger I, which is similar to WTX [97,99]. Candoxin blocks muscle and neuronal α7 nAChR. IC50 on rat α1β1γδ ε nAChR expressed in oocytes is 10 nM, on rat α7 is 50 nM. The inhibition by candoxin is reversible for mouse-type of receptors, and poorly reversible for α7 nAChR. LD50 of candoxin is 0.83 mg/kg, which is higher than of WTX [97].

3.5. Toxins with Unique Structure

Denmotoxin is a 77-amino-acid-long toxin isolated from Boiga dendrophila snake, with a unique property to discriminate between its targets. It irreversibly blocks chick αβδγ ε nAChR, while it is a 100-fold weaker antagonist of mouse muscle nAChR [132]. Injected intraperitoneally to mice at 20 mg/kg, no biological effect was observed. Structurally, it possesses five disulfide bonds, with an extra disulfide bond within loop I of the toxin.

Lc-a and Lc-b are both 69 amino-acid-long toxins isolated from sea snake Laticauda colubrina [133]. They both contain four disulfide bonds and are lethal when injected intramuscularly to mice with an LD50 of 0.12 mg/kg.

Ω-Neurotoxin Oh-9 is a 57 amino-acid-long toxin isolated from Ophiophagus Hannah, with four disulfide bonds and a low sequence similarity with α-neurotoxins of snake origin [134]. The toxin inhibits rat muscle α1β1δ ε nAChRs, as well as α3β2 receptor subtype. Mutagenesis studies revealed that the tip of loop II is not crucial for binding. Met 25 and Phe 27 constitute the binding core of the toxin to both muscle and neuronal receptor types, whereas Thr 23 and Phe 26 are critical for interaction with α1β1δ ε but not with α3β2 [135].

αδ-bungarotoxin-1 is 73 amino-acid-long toxin isolated from Bungarus candidus, with five disulfide bonds and inhibitory activity on muscle and neuronal α7 nAChRs. Unlike α-Bgtx, αδ-Bgtx binds to the target receptor in a reversible manner. Binding affinities are higher at the α-δ interface than at the α-γ or α-ε interfaces [117]. The reason for such selectivity could be due to a shorter finger I, and thus a smaller number of contacts with

Table 2. Cont.

| Toxin Same (UniProt) | Snake Species | Target (IC50 or Kd Values) | Ref. |
|----------------------|---------------|---------------------------|-----|
| Lc-a (P0C8R7)        | Yellow-lipped sea krait Laticauda colubrina (Elapidae) | Muscle nAChR LD50 = 0.12 µg/g following intramuscular injection in mice | [133] |
| Lc-b (P0C8R8)        |               |                           |     |
| Neurotoxin Oh-9 (P83302) | King cobra Ophiophagus hannah (Elapidae) | IC50 on carbachol-induced chicken cervicis muscle contractions—88 nM;画   Rat adult muscle (IC50 = 3.1 µM); Rat fetal muscle (IC50 = 5.6 µM) | [134,135] |
| Waglerin-1 (P24335)  | Wagler’s palm viper Trimeresurus wagleri (Viperidae) | max1β1δ ε (IC50 = 50 nM, end-plate potential inhibition);画   max1β1γδ ε (IC50 = 36 µM);画   2000-fold higher affinity to the α-ε (Kd = 9.8 nM) than to the α-δ (Kd = 20.2 µM) binding site interface of the mouse muscle receptor;画   hα1β1δ ε (Kd1 = 692 nM, Kd2 = 200 µM)画   rα1β1δ ε (Kd1 = 1.1 µM, Kd2 = 36 µM) | [136–138] |
the receptor interface, as well fewer positively charged amino acids within finger II of the toxin.

Waglerin-1 is 22 amino-acid-long peptide with a single disulfide bond. Despite its difference in length and structural features with other snake neurotoxins, Waglerin-1 blocks the muscle nAChR containing ε subunit \[136,140\], and has a 2000-fold higher affinity for α-ε compared to the α-δ subunit interface. The toxin is not potent on the fetal muscle receptor containing the γ subunit \[136\]. Besides its activity on nAChRs, Waglerin-1 also potentiates and suppresses GABA<sub>A</sub> receptors (EC<sub>50</sub> = 24 µM) \[137\].

4. Peptides of Spider Venom Origin as Potential Ligands Acting on nAChRs

Spiders do not possess a diverse arsenal of toxins which act on acetylcholine receptors. This fact is surprising when considering the importance of acetylcholine receptors for insects, the main prey of spiders. The action of spider toxins on nicotinic receptors is observed, alongside their activity on other targets.

The toxin HWTX-1 (Huwentoxin-1), from a Chinese bird spider *Selenocosmia huwena* (synonymous to *Haplopelma schmidti*) \[141,142\], is a 33 amino-acid-long peptide with six cysteines connected in a following way: C1–C4; C2–C5; C3–C6, which is similar to disulfide bonds of ω-conotoxin GVIA. HWTX-1 was initially shown to be potent in inhibiting neuromuscular transmission and evoking muscle paralysis. At 10 µg/mL, the blockade of twitch response was achieved in 15.2 min in a mouse phrenic nerve-hemidiaphragm preparation \[142\]. Later, however, the effect of the toxin on acetylcholine receptors was questioned \[143–145\]. Now, it is established that the main target of the HWTX-I is the N-type Calcium channel (IC<sub>50</sub> is 100 nM) \[144\].

Another toxin of spider origin with potential activity on nicotinic acetylcholine receptors is ω-Agatoxin IVA, derived from the American funnel spider, *Agelenopsis aperta*. The inhibitory activity of the toxin was demonstrated on bovine chromaffin cells, where the toxin at a 100 nM concentration inhibited nicotine-evoked current \[146\]. However, besides this, the toxin was not shown to be active on nicotinic receptors. The main mechanism of its action is through the blockage of P-type voltage-gated calcium channels \[147,148\].

The only peptidic toxin of spider origin that was shown to affect nicotinic receptors is κ-Hexatoxin Hv1c (κ-HXTX-Hv1C), from the blue-mountain funnel web spider, *Hadronyche versuta* \[149\]. It acts as a positive allosteric modulator of the receptors (Table 3).

| Table 3. The toxins of spider origin, with a suggested and demonstrated activity on nAChRs. |
|---------------------------------|---------------------------------|-----------------|---|
| Toxin Name/Spider Species/UniProt | Amino-Acid Sequence | Ref. |
|---------------------------------|---------------------------------|-----------------|---|
| HWTX-I *Selenocosmia huwena* P56676 | ACKGVFDACTPGKNECCPNRVCSDKHKWCKWK | \[142\] |
| ω-agatoxin IVA *Agelenopsis aperta* P30288 | KKKCIAKDYGRCKWGTPCCRGRGCCSIMGTNCECPRLIMEGLLA | \[146\] |
| κ-HXTX-Hv1c *Hadronyche versuta* P82228 | AICTGADRPCAACCPCPGTSCKAESNGVSYCRKDEP | \[149\] |

5. Peptides of Scorpion Venom Origin as Potential Ligands Acting on nAChRs

Scorpion venom is a rich source of ligands targeting voltage-gated potassium (K<sub>v</sub>) and sodium (Nav) channels \[150,151\]. The affinity for these channels is at nM and even at pM range. Scorpion toxins are classified into several families and subfamilies based on four criteria. The criteria include the type of affected ion channel (sodium, potassium, calcium, or chloride) \[152\], target site on the channel, three-dimensional structure, and type of response. The Nav-targeting toxins are divided into α- and β- type depending on the binding site on the channels and the resultant effect of binding. α-toxins bind at site 3 and
inhibit channel inactivation, while β toxins bind at site 4 and change channel activation. The Kv-targeting toxins are divided into α-, β-, γ-, and κ- families. κ- family toxins have α-helix-loop-helix fold, where two short α-helices are connected by a β-turn [152]. A recent report by Kasheverov [153] showed that scorpion peptides demonstrate target promiscuity (Table 4). Thus, scorpion toxins within α-KTx and κ-KTX families target nicotinic AChRs of muscle and α7 type, with a higher affinity towards muscle-type receptor vs. homomeric neuronal α7 type. For example, the IC50 of HelaTx1 on *Torpedo californica* nAChRs is 1.4 ± 0.1 µM; on human, α7 nAChR is 64 ± 7 µM (Table 4). HelaTx1 is a toxin within the κ-KTx family with two short α-helices stabilized by two disulfide bonds; by structure, it resembles α-conotoxins. Surprisingly, it has a higher affinity towards muscle-type nAChRs compared to the Kv channel. Overall, affinities towards the nAChRs for scorpion toxins are in µM range, which makes it unlikely that nAChRs are the main biological targets for scorpion venom.

6. Conclusions

Natural products provide a rich source of ligands targeting nAChRs. The venom of snakes and *Cone* snails is especially rich in toxins that have a high affinity (nM range) and selectivity towards nAChRs. For example, α-conotoxins with 3/5 spacing between disulfide bridges, as well as long-chain three-finger α-neurotoxins of snake origin, are potent inhibitors of muscle-type nAChRs. But α-conotoxins with 4/7 spacing that target neuronal types of nAChR often demonstrate similarly high affinities towards multiple kinds of heteromeric receptors. The selectivity of toxins targeting multiple types of receptors could be improved, however. α-conotoxin MII [H9A; L15A], for example, demonstrates selectivity towards α6-containing receptors; ArIB [V11L; V16D] is a selective α7 nAChR antagonist, while PeIA [S9H; V10A; E14N] can discriminate between β2 and β4 receptor subunits in α6-containing receptors. Novel toxins of venomous origin hold great potential for the development of highly potent and selective ligand targeting nAChRs, and they could be extensively used in research.

### Funding

This research was funded by Nazarbayev University (NU) Faculty Development Competitive Research Grant, grant number 110119FD4517.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Acknowledgments:** The authors would like to acknowledge Yunona Bukasova for assistance with Figure 1 and proofreading the manuscript.

**Conflicts of Interest:** The authors declare no conflict of interest.

### References

1. Albuquerque, E.X.; Pereira, E.F.R.; Alkondon, M.; Rogers, S.W. Mammalian Nicotinic Acetylcholine Receptors: From Structure to Function. Physiol. Rev. 2009, 89, 73–120. [CrossRef] [PubMed]
2. Changeux, J.-P. Discovery of the First Neurotransmitter Receptor: The Acetylcholine Nicotinic Receptor. Biomolecules 2020, 10, 547. [CrossRef]
3. Millar, N.S.; Gotti, C. Diversity of Vertebrate Nicotinic Acetylcholine Receptors. Neuropharmacology 2009, 56, 237–246. [CrossRef]
27. Liu, L.; Chew, G.; Hawrot, E.; Chi, C.; Wang, C. Two Potent A3/5 Conotoxins from Piscivorous Conus Achatinus. *Front. Synaptic Neurosci.* 2020, 12, 54. [CrossRef]

28. Groebe, D.R.; Dumm, J.M.; Levitan, E.S.; Abramson, S.N. Alpha-Conotoxins Selectively Inhibit One of the Two Acetylcholine Binding Sites Crucial for Activity at the Muscle Acetylcholine Receptor. *Mar. Drugs* 2015, 12, 10905. [CrossRef] [PubMed]

29. Plazas, P.V.; Katz, E.; Gomez-Casati, M.E.; Bouzat, C.; Elgoyhen, A.B. Stoichiometry of the A9x10 Nicotinic Cholinergic Receptor. *J. Neurosci.* 2005, 25, 10905. [CrossRef] [PubMed]

30. Gharpure, A.; Noviello, C.M.; Hibbs, R.E. Progress in Nicotinic Receptor Structural Biology. *Neuropharmacology* 2020, 171, 108086. [CrossRef] [PubMed]

31. Karlin, A. A Touching Picture of Nicotinic Binding. *Neuron* 2004, 41, 841–842. [CrossRef]

32. Kumbhare, D.; Muttenthaler, M.; Dutertre, S.; Himaya, S.W.A.; Kaas, Q.; Craik, D.J.; Lewis, R.J.; Alewood, P.F. Conotoxins: Chemistry and Biology. *Front. Synaptic Neurosci.* 2015, 8, 399–407. [CrossRef]

33. Bencherif, M.; Lippiello, P.M.; Lucas, R.; Marrero, M.B. Alpha7 Nicotinic Receptors as Novel Therapeutic Targets for Inflammation-Based Diseases. *Cell. Mol. Life Sci.* 2011, 68, 931–949. [CrossRef]

34. Winek, K.; Soreq, H.; Meisel, A. Regulators of Cholinergic Signaling in Disorders of the Central Nervous System. *J. Neurochem.* 2021. [CrossRef]

35. Dineley, K.T.; Pandya, A.A.; Yakel, J.L. Nicotinic ACh Receptors as Therapeutic Targets in CNS Disorders. *Trends Pharmacol. Sci.* 2015, 36, 96–108. [CrossRef] [PubMed]

36. Quik, M.; Zhang, D.; McGregor, M.; Bordia, T. Alpha7 Nicotinic Receptors as Therapeutic Targets for Parkinson’s Disease. *Biochem. Pharmacol.* 2015, 97, 399–407. [CrossRef] [PubMed]

37. Pennington, M.W.; Czerwinski, A.; Norton, R.S. Peptide Therapeutics from Venom: Current Status and Potential. *Bioorgan. Med. Chem.* 2018, 26, 2738–2758. [CrossRef] [PubMed]

38. Jin, A.-H.; Muttenthaler, M.; Dutertre, S.; Himaya, S.W.A.; Kaas, Q.; Craik, D.J.; Lewis, R.J.; Alewood, P.F. Conotoxins: Chemistry and Biology. *Chem. Rev.* 2019, 119, 11510–11549. [CrossRef] [PubMed]

39. Abraham, N.; Lewis, R.J. Neuronal Nicotinic Acetylcholine Receptor Modulators from Cone Snails. *Mar. Drugs* 2018, 16, 208. [CrossRef] [PubMed]

40. Giribaldi, J.; Dutertre, S. α-Conotoxins to Explore the Molecular, Physiological and Pathophysiological Functions of Neuronal Nicotinic Acetylcholine Receptors. *Neurosci. Lett.* 2018, 679, 24–34. [CrossRef] [PubMed]

41. McIntosh, J.M.; Santos, A.D.; Olivera, B.M. Conus Peptides Targeted to Specific Nicotinic Acetylcholine Receptor Subtypes. *Annu. Rev. Biochem.* 1999, 68, 59–88. [CrossRef] [PubMed]

42. Jacobson, K.B.; DelaCruz, R.G.; Grose, J.H.; McIntosh, J.M.; Yoshikami, D.; Olivera, B.M. Critical Residues Influence the Affinity and Selectivity of α-Conotoxin MI for Nicotinic Acetylcholine Receptors. *Biochemistry* 1999, 38, 13310–13315. [CrossRef] [PubMed]

43. Liu, L.; Chew, G.; Hawrot, E.; Chi, C.; Wang, C. Two Potent A3/A5 Conotoxins from Piscivorous Conus Achatinus. *Acta Biochim. Biophys. Sin.* 2007, 39, 438–444. [CrossRef] [PubMed]

44. Groebe, D.R.; Dumm, J.M.; Levitan, E.S.; Abramson, S.N. Alpha-Conotoxins Selectively Inhibit One of the Two Acetylcholine Binding Sites of Nicotinic Receptors. *Mol. Pharmacol.* 1995, 48, 105.

45. Groebe, D.R.; Gray, W.R.; Abramson, S.N. Determinants Involved in the Affinity of α-Conotoxin GI and SI for the Muscle Subtype of Nicotinic Acetylcholine Receptors. *Biochemistry* 1997, 36, 6469–6474. [CrossRef] [PubMed]

46. Ning, J.; Li, R.; Ren, J.; Zhangsun, D.; Zhu, X.; Wu, Y.; Luo, S. Alanine-Scanning Mutagenesis of α-Conotoxin GI Reveals the Residues Crucial for Activity at the Muscle Acetylcholine Receptor. *Mar. Drugs* 2018, 16, 507. [CrossRef] [PubMed]
31. Giribaldi, J.; Wilson, D.; Nicke, A.; El Hamdaoui, Y.; Laconde, G.; Faucherre, A.; Moha Ou Maati, H.; Daly, N.L.; Enjalbal, C.; Dutertre, S. Synthesis, Structure and Biological Activity of CIA and CIB, Two α-Conotoxins from the Predation-Evoked Venom of Conus Catus. Toxins 2018, 10, 222. [CrossRef]
32. Ramilo, C.A.; Zafarulla, G.C.; Nadasdi, L.; Hammerland, L.G.; Yoshikami, D.; Gray, W.R.; Kristipati, R.; Ramachandran, J.; Miljanich, G. Novel Alpha.- and Omega.-Conotoxins and Conus Striatus Venom. Biochemistry 1992, 31, 9919–9926. [CrossRef] [PubMed]
33. Quiram, P.A.; Sine, S.M. Structural Elements in α-Conotoxin IIm Essential for Binding to Neuronal A7 Receptors. J. Biol. Chem. 1998, 273, 11007–11011. [CrossRef] [PubMed]
34. Ellison, M.; McIntosh, J.M.; Olivera, B.M. α-Conotoxins Iml and Iml: Similar A7 nicotinic receptor antagonists act at different sites. J. Biol. Chem. 2003, 278, 757–764. [CrossRef]
35. Ellison, M.; Feng, Z.-P.; Park, A.J.; Zhang, X.; Olivera, B.M.; McIntosh, J.M.; Norton, R.S. α-RgIA, a Novel Conotoxin That Blocks the A9α10 NAcHR: Structure and Identification of Key Receptor-Binding Residues. J. Mol. Biol. 2008, 377, 1216–1227. [CrossRef]
36. Chhabra, S.; Belgi, A.; Bartels, P.; van Lierop, B.J.; Robinson, S.D.; Kompella, S.N.; Hung, A.; Callaghan, B.P.; Adams, D.J.; Robinson, A.J.; et al. Dicarba Analogues of α-Conotoxin RgIA. Structure, Stability, and Activity at Potential Pain Targets. J. Med. Chem. 2014, 57, 9933–9944. [CrossRef] [PubMed]
37. Azam, L.; Dowell, C.; Watkins, M.; Stitzel, J.A.; Olivera, B.M.; McIntosh, J.M. α-Conotoxin BuA, a Novel Peptide from Conus Bullatus, Distinguishes among Neuronal Nicotinic Acetylcholine Receptors. J. Biol. Chem. 2005, 280, 80–87. [CrossRef] [PubMed]
38. Kim, H.-W.; McIntosh, J.M. A6 NACHR Subunit Residues That Confer α-Conotoxin BuA Selectivity. FASEB J. 2012, 26, 4102–4110. [CrossRef]
39. Azam, L.; Maskos, U.; Changeux, J.-P.; Dowell, C.D.; Christensen, S.; De Biasi, M.; McIntosh, J.M. α-Conotoxin BuA[5A,6P6O]: A Novel Ligand That Discriminates between A6β4 and A6β2 Nicotinic Acetylcholine Receptors and Blocks Nicotine-Stimulated Norepinephrine Release. FASEB J. 2010, 24, 5113–5123. [CrossRef] [PubMed]
40. Quinton, L.; Servent, D.; Girard, E.; Molgo, J.; Le Caer, J.-P.; Malosse, C.; Haidar, E.A.; Lecooq, A.; Gilles, N.; Chomat-Rooke, J. Identification and Functional Characterization of a Novel α-Conotoxin (EIIA) from Conus Ermineus. Anal. Bioanal. Chem. 2013, 405, 5341–5351. [CrossRef] [PubMed]
41. Grishin, A.A.; Cuny, H.; Hung, A.; Clark, R.J.; Brust, A.; Akondi, K.; Alewood, P.F.; Craik, D.J.; Adams, D.J. Identifying Key Amino Acid Residues That Affect α-Conotoxin AuIβ Inhibition of Aβ34 Nicotinic Acetylcholine Receptors. J. Biol. Chem. 2013, 288, 34428–34442. [CrossRef]
42. Li, L.; Liu, N.; Ding, R.; Wang, S.; Liu, Z.; Li, H.; Zheng, X.; Dai, Q. A Novel 4/6-Type Alpha-Conotoxin ViIA Selectively Inhibits NAcHR A3β2 Subtype. Acta Biochim. Biophys. Sin. 2015, 47, 1023–1028. [CrossRef] [PubMed]
43. van Hout, M.; Valdes, A.; Christensen, S.B.; Tran, P.T.; Watkins, M.; Gajewiak, J.; Jensen, A.A.; Olivera, B.M.; McIntosh, J.M. α-Conotoxin VnIB from Conus Ventricosus Is a Potent and Selective Antagonist of A6β4* Nicotinic Acetylcholine Receptors. Neuropharmacology 2019, 157, 107691. [CrossRef] [PubMed]
44. Wu, Y.; Zhangsun, D.; Zhu, X.; Kaas, Q.; Zhangsun, M.; Harvey, P.J.; Craik, D.J.; McIntosh, J.M.; Luo, S. α-Conotoxin [S9A]Tx1D Potently Discriminates between A3β4 and A6/34 Nicotinic Acetylcholine Receptors. J. Med. Chem. 2017, 60, 5826–5833. [PubMed]
45. Loughnan, M.L.; Nicke, A.; Jones, A.; Adams, D.J.; Alewood, P.F.; Lewis, R.J. Chemical and Functional Identification and Characterization of Novel Sulfated α-Conotoxins from the Cone Snail Conus Anemone. J. Med. Chem. 2004, 47, 1234–1241. [CrossRef]
46. Whiteaker, P.; Christensen, S.; Yoshikami, D.; Dowell, C.; Watkins, M.; Gulyas, J.; Rivier, J.; Olivera, B.M.; McIntosh, J.M. Discovery, Synthesis, and Structure Activity of a Highly Selective A7 Nicotinic Acetylcholine Receptor Antagonist. Biochemistry 2007, 46, 6628–6638. [CrossRef]
47. Ning, J.; Ren, J.; Xiong, Y.; Wu, Y.; Zhangsun, M.; Zhangsun, D.; Zhu, X.; Luo, S. Identification of Crucial Residues in α-Conotoxin El Inhibiting Muscle Nicotinic Acetylcholine Receptor. Toxins 2019, 11, 603. [CrossRef]
48. Lin, B.; Xu, M.; Zhu, X.; Wu, Y.; Liu, X.; Zhangsun, D.; Hu, Y.; Xiang, S.-H.; Kashchevorov, I.E.; Tsetlin, V.I.; et al. From Crystal Structure of α-Conotoxin GIC in Complex with Ac-AChBP to Molecular Determinants of Its High Selectivity for A3β2 NACHR. Sci. Rep. 2016, 6, 22349. [CrossRef]
49. Banerjee, J.; Yongye, A.B.; Chang, Y.-P.; Gyanda, R.; Medina-Franco, J.L.; Armishaw, C.J. Design and Synthesis of α-Conotoxin GID Analogues as Selective A4β2 Nicotinic Acetylcholine Receptor Antagonists. Biopolymers 2014, 102, 78–87. [CrossRef]
50. Lisette, E.K.M.; Peigneur, S.; Maiti, M.; Devi, P.; Ravichandran, S.; Lescrinier, E.; Ullens, C.; Waelsens, E.; D’Souza, L.; Herdevijn, P.; et al. Structure-Function Elucidation of a New α-Conotoxin, Lo1a, from Conus Longurionis. J. Biol. Chem. 2014, 289, 9573–9583. [PubMed]
51. Inseri, M.C.; Kompella, S.N.; Vetter, I.; Brust, A.; Daly, N.L.; Cuny, H.; Craik, D.J.; Alewood, P.F.; Adams, D.J.; Lewis, R.J. Isolation and Characterization of α-Conotoxin LsIA with Potent Activity at Nicotinic Acetylcholine Receptors. Biochem. Pharmacol. 2013, 86, 791–799. [CrossRef] [PubMed]
52. Chen, J.; Liang, L.; Ning, H.; Cai, F.; Liu, Z.; Zhang, L.; Zhou, L.; Dai, Q. Cloning, Synthesis and Functional Characterization of a Novel α-Conotoxin Ll13. Mar. Drugs 2018, 16, 112. [CrossRef]
53. Luo, S.; Zhangsun, D.; Schroeder, C.I.; Zhu, X.; Hu, Y.; Wu, Y.; Weltzin, M.M.; Eberhard, S.; Kaas, Q.; Craik, D.J.; et al. A Novel A4/7-Conotoxin LvIA from Conus Lividus That Selectively Blocks A3β2 vs. A6/A3β2β3 Nicotinic Acetylcholine Receptors. *FASEB J.* 2014, 28, 1842–1853. [CrossRef] [PubMed]

54. Xu, M.; Zhu, X.; Yu, J.; Yu, J.; Luo, S.; Wang, X. The Crystal Structure of Ac-AChBP in Complex with α-Conotoxin LvIA Reveals the Mechanism of Its Selectivity towards Different NACHR Subtypes. *Protein Cell* 2017, 8, 675–685. [CrossRef] [PubMed]

55. Everhart, D.; Cartier, G.E.; Malhotra, A.; Gomes, A.V.; McIntosh, J.M.; Luetje, C.W. Determinants of Potency on Alpha-Conotoxin MII, a Peptide Antagonist of Neuronal Nicotinic Receptors. *Biochemistry* 2004, 43, 2732–2737. [CrossRef]

56. McIntosh, J.M.; Azam, L.; Staheli, S.; Dowell, C.; Lindstrom, J.M.; Kuryatov, A.; Garrett, J.E.; Marks, M.J.; Whiteaker, P. Analogs of Alpha-Conotoxin MII Are Selective for Alpha6-Containing Nicotinic Acetylcholine Receptors. *Mol. Pharmacol.* 2004, 65, 944–952. [CrossRef] [PubMed]

57. Wang, S.; Zhao, C.; Liu, Z.; Wang, X.; Liu, N.; Du, W.; Dai, Q. Structural and Functional Characterization of a Novel α-Conotoxin Mr1.7 from Conus Marmoreus Targeting Neuronal NACHR A3β2, A9x10 and A6/A3β2β3 Subtypes. *Mar. Drugs* 2015, 13, 3259–3275. [CrossRef] [PubMed]

58. Hone, A.J.; Ruiz, M.; Scadden, M.; Christensen, S.; Gajewiak, J.; Azam, L.; McIntosh, J.M. Positional Scanning Mutagenesis of α-Conotoxin PeIA Identifies Critical Residues That Confer Potency and Selectivity for A6/A3β2β3 and A3β2 Nicotinic Acetylcholine Receptors. *J. Biol. Chem.* 2013, 288, 25428–25439. [CrossRef]

59. Hone, A.J.; Fisher, F.; Christensen, S.; Gajewiak, J.; Larkin, D.; Whiteaker, P.; McIntosh, J.M. PeIA-5466: A Novel Peptide Antagonist Containing Non-Natural Amino Acids That Selectively Targets A3β2 Nicotinic Acetylcholine Receptors. *J. Med. Chem.* 2019, 62, 6262–6275. [CrossRef]

60. Hone, A.J.; Scadden, M.; Gajewiak, J.; Christensen, S.; Lindstrom, J.; McIntosh, J.M. α-Conotoxin PeIA[S9H,V10A,E14N] Potently and Selectively Blocks A6β2β3 versus A6β4 Nicotinic Acetylcholine Receptors. *Mol. Pharmacol.* 2012, 82, 972–982. [CrossRef]

61. Luo, S.; Nguyen, T.A.; Cartier, G.E.; Olivera, B.M.; Yoshikami, D.; McIntosh, J.M. Single-Residue Alteration in α-Conotoxin PnIA Switches Its NACHR Subtype Selectivity. *Biochemistry* 1999, 38, 14542–14548. [CrossRef] [PubMed]

62. Hogg, R.C.; Hopping, G.; Alewood, P.F.; Adams, D.J.; Bertrand, D. Alpha-Conotoxins PnIA and [A10L]PnIA Stabilize Different States of the Alpha7-L247T Nicotinic Acetylcholine Receptor. *J. Biol. Chem.* 2003, 278, 26908–26914. [CrossRef]

63. Kompella, S.N.; Hung, A.; Clark, R.J.; Mari, F.; Adams, D.J. Alanine Scan of α-Conotoxin RegIIA Reveals a Selective A3β4 Nicotinic Acetylcholine Receptor Antagonist. *J. Biol. Chem.* 2015, 290, 1039–1048. [CrossRef]

64. Luo, S.; Zhangsun, D.; Zhu, X.; Wu, Y.; Hu, Y.; Christensen, S.; Harvey, P.J.; Akcan, M.; Craik, D.J.; McIntosh, J.M. Characterization of a Novel α-Conotoxin TxID from Conus Textile That Potentially Blocks Rat A3β4 Nicotinic Acetylcholine Receptors. *J. Med. Chem.* 2013, 56, 9655–9663. [CrossRef]

65. Jin, A.-H.; Vetter, I.; Dutertre, S.; Abraham, N.; Emidio, N.B.; Inserra, M.; Murali, S.S.; Christie, M.J.; Alewood, P.F.; Lewis, R.J. Mr1C, a Novel α-Conotoxin Agonist in the Presence of PNU at Endogenous A7 Nicotinic Acetylcholine Receptors. *Biochemistry* 2014, 53, 1–6. [CrossRef]

66. Halai, R.; Clark, R.J.; Nevin, S.T.; Jensen, J.E.; Adams, D.J.; Craik, D.J. Scanning Mutagenesis of Alpha-Conotoxin Vc1.1 Reveals Residues Crucial for Activity at the Alpha9alpha10 Nicotinic Acetylcholine Receptor. *J. Biol. Chem.* 2009, 284, 20275–20284. [CrossRef]

67. Dutton, J.L.; Craik, D.J. α-Conotoxin Antagonists: Nicotinic Acetylcholine Receptor Antagonists as Pharmacological Tools and Potential Drug Leads. *Curr. Med. Chem.* 2001, 8, 327–344. [CrossRef]

68. Halai, R.; Craik, D.J. Conotoxins: Natural Product Drug Leads. *Nat. Prod. Rep.* 2009, 26, 526–536. [CrossRef] [PubMed]

69. Li, X.; Tae, H.-S.; Chu, Y.; Jiang, T.; Adams, D.J.; Yu, R. Medicinal Chemistry, Pharmacology, and Therapeutic Potential of α-Conotoxins Antagonizing the A9α10 Nicotinic Acetylcholine Receptor. *Pharmacol. Ther.* 2020, 222, 107792. [CrossRef]

70. Di Cesare Mannelli, L.; Cinci, L.; Micheli, L.; Zanardelli, M.; Pacini, A.; McIntosh, J.M.; Ghelardini, C. α-Conotoxin RgIA Protects against the Development of Nerve Injury-Induced Chronic Pain and Prevents Both Neuronal and Glial Derangement. *Pain* 2014, 155, 1866–1876. [CrossRef]

71. Hone, A.J.; Servent, D.; McIntosh, J.M. A9-Containing Nicotinic Acetylcholine Receptors and the Modulation of Pain. *Br. J. Pharmacol.* 2018, 175, 1915–1927. [CrossRef]

72. Mohammadi, S.; Christie, M.J. A9-Nicotinic Acetylcholine Receptors Contribute to the Maintenance of Chronic Mechanical Hyperalgesia, but Not Thermal or Mechanical Allodynia. *Mol. Pain* 2014, 10, 1744–8069. [CrossRef]

73. Mohammadi, S.A.; Christie, M.J. Conotoxin Interactions with A9x10-NACHRs: Is the A9x10 Nicotinic Acetylcholine Receptor an Important Therapeutic Target for Pain Management? *Toxins* 2015, 7, 9396–9392. [CrossRef] [PubMed]

74. Vincler, M.; McIntosh, J.M. Targeting the A9α10 Nicotinic Acetylcholine Receptor to Treat Severe Pain. *Expert Opin. Ther. Targets* 2007, 11, 891–897. [CrossRef] [PubMed]

75. Satkunanathan, N.; Livett, B.; Gayler, K.; Sandall, D.; Down, J.; Khalil, Z. Alpha-Conotoxin Vc1.1 Alleviates Neuropathic Pain and Accelerates Functional Recovery of Injured Neurons. *Brain Res.* 2005, 1059, 149–158. [CrossRef]

76. Romero, H.K.; Christensen, S.B.; Di Cesare Mannelli, L.; Gajewiak, J.; Ramachandra, R.; Elmslie, K.S.; Vetter, D.E.; Ghelardini, C.; Iadonato, S.P.; Mercado, J.L.; et al. Inhibition of A9α10 Nicotinic Acetylcholine Receptors Prevents Chemotherapy-Induced Neuropathic Pain. *Proc. Natl. Acad. Sci. USA* 2017, 114, E1825–E1832. [CrossRef]
100. Osipov, A.V.; Kasheverov, I.E.; Makarova, Y.V.; Starkov, V.G.; Vorontsova, O.V.; Ziganšin, R.K.; Andreeva, T.V.; Serebryakova, M.V.; Benoit, A.; Hogg, R.C.; et al. Naturally Occurring Disulfide-Bound Dimers of Three-Fingered Toxins. *J. Biol. Chem.* 2008, 283, 14571–14580. [CrossRef] [PubMed]

101. Osipov, A.V.; Rucktoo, P.; Kasheverov, I.E.; Filkin, S.Y.; Starkov, V.G.; Andreeva, T.V.; Sixma, T.K.; Bertrand, D.; Utkin, Y.N.; Tsetlin, V.I. Dimeric α-Cobratoxin X-ray structure: Localization of intermolecular disulfides and possible mode of binding to nicotinic acetylcholine receptors. *J. Biol. Chem.* 2012, 287, 6725–6734. [CrossRef] [PubMed]

102. Foo, C.S.; Jobichen, C.; Hassan-Puttaswamy, V.; Dekan, Z.; Tae, H.-S.; Bertrand, D.; Adams, D.J.; Alewood, P.F.; Sivaraman, J.; Nirthanan, S.; et al. Fulidotoxin, Representing a New Class of Dimeric Snake Toxins, Defines Novel Pharmacology at Nicotinic ACh Receptors. *Br. J. Pharmacol.* 2020, 177, 1822–1840. [CrossRef] [PubMed]

103. Pawlak, J.; Mackessy, S.P.; Sixberry, N.M.; Stura, E.A.; Le Du, M.H.; M.

104. Nirthanan, S. Snake Three-Finger α-Neurotoxins and Nicotinic Acetylcholine Receptors: Molecules, Mechanisms and Medicine. *Biochem. Pharmacol.* 2020, 181, 114168. [CrossRef] [PubMed]

105. Chang, C.C. Looking Back on the Discovery of α-Bungarotoxin. *J. Biomed. Sci.* 1999, 6, 368–375. [CrossRef]

106. Changeux, J.P.; Kasai, M.; Lee, C.Y. Use of a Snake Venom Toxin to Characterize the Cholinergic Receptor Protein. *Proc. Natl. Acad. Sci. USA* 1970, 67, 1241–1247. [CrossRef]

107. Wang, G.K.; Schmidt, J. Primary Structure and Binding Properties of Iodinated Derivatives of Alpha-Bungarotoxin. *J. Biol. Chem.* 1980, 255, 11156–11162. [CrossRef]

108. Servent, D.; Mourier, G.; Antil, S.; Ménez, A. How Do Snake Curaremimetic Toxins Discriminate between Nicotinic Acetylcholine Receptor Subtypes. *Toxicol. Lett.* 1998, 102–103, 199–203. [CrossRef]

109. Moise, L.; Piserchio, A.; Basus, V.J.; Hawrot, E. NMR Structural Analysis of α-Bungarotoxin and Its Complex with the Principal α-Neurotoxin-Binding Sequence on the A7 Subunit of a Neuronal Nicotinic Acetylcholine Receptor. *J. Biol. Chem.* 2002, 277, 12406–12417. [CrossRef]

110. Dellissanti, C.D.; Yao, Y.; Stroud, J.C.; Wang, Z.-Z.; Chen, L. Crystal Structure of the Extracellular Domain of NACHr A1 Bound to α-Bungarotoxin at 1.94 Å Resolution. *Nat. Neurosci.* 2007, 10, 953–962. [CrossRef]

111. Huang, S.; Li, S.-X.; Bren, N.; Cheng, K.; Gomoto, R.; Chen, L.; Sine, S.M. Complex between α-Bungarotoxin and an A7 Nicotinic Receptor Ligand-Binding Domain Formica. *Biochem. J.* 2013, 454, 303–310. [CrossRef] [PubMed]

112. Zouridakis, M.; Giastas, P.; Zarkadas, E.; Chroni-Tzartou, D.; Bregestovski, P.; Tzartos, S.J. Crystal Structures of Free and Antagonist-Bound States of Human A9 Nicotinic Acetylcholine Receptor Extracellular Domain. *Nat. Struct. Mol. Biol.* 2014, 21, 976–980. [CrossRef]

113. Rahman, M.M.; Teng, J.; Worrell, B.T.; Noviello, C.M.; Lee, M.; Karlin, A.; Stowell, M.H.B.; Hibbs, R.E. Structure of the Native Muscle-Type Nicotinic Receptor and Inhibition by Snake Venom Toxins. *Neuron* 2020, 106, 952–962.e5. [CrossRef]

114. Antil-Delbeke, S.; Gaillard, C.; Tamiya, T.; Corringer, P.-J.; Changeux, J.-P.; Servent, D.; Ménez, A. Molecular Determinants by Which a Long Chain Toxin from Snake Venom Interacts with the Neuronal A7-Nicotinic Acetylcholine Receptor. *J. Biol. Chem.* 2000, 275, 29594–29601. [CrossRef]

115. Fruchart-Gaillard, C.; Gilquin, B.; Antil-Delbeke, S.; Le Novère, N.; Tamiya, T.; Corringer, P.-J.; Changeux, J.-P.; Ménez, A.; Servent, D. Experimentally Based Model of a Complex between a Snake Toxin and the A7 Nicotinic Receptor. *Proc. Natl. Acad. Sci. USA* 2002, 99, 3216. [CrossRef]

116. Bourne, Y.; Talley, T.T.; Hansen, S.B.; Taylor, P.; Marchot, P. Crystal Structure of a Ctba–ACHBP Complex Reveals Essential Interactions between Snake α-Neurotoxins and Nicotinic Receptors. *EMBO J.* 2005, 24, 1512–1522. [CrossRef]

117. Utkin, Y.N.; Kuch, U.; Kasheverov, I.E.; Lebedev, D.S.; Cederlund, E.; Molles, B.E.; Polyak, I.; Ivanov, I.A.; Prokopev, N.A.; Ziganšin, R.H.; et al. Novel Long-Chain Neurotoxins from Bungarus Candidus Distinguish the Two Binding Sites in Muscle-Type Nicotinic Acetylcholine Receptors. *Biochem. J.* 2019, 476, 1285–1302. [CrossRef]

118. Chandna, R.; Tae, H.-S.; Seymour, V.A.L.; Chathrath, S.; Adams, D.J.; Kini, R.M. Drysdalin, an Antagonist of Nicotinic Acetylcholine Receptors Highlights the Importance of Functional Rather than Structural Conservation of Amino Acid Residues. *FASEB J.* 2019, 1, 115–131. [CrossRef]

119. Servent, D.; Winckler-Dietrich, V.; Hu, H.-Y.; Kessler, P.; Drevet, P.; Bertrand, D.; Ménez, A. Only Snake Curaremimetic Toxins with a Fifth Disulfide Bond Have High Affinity for the Neuronal A7 Nicotinic Receptor. *J. Biol. Chem.* 1997, 272, 24279–24286. [CrossRef] [PubMed]

120. Trémeau, O.; Lemaire, C.; Drevet, P.; Pinkasfeld, S.; Ducancel, F.; Boulain, J.-C.; Ménez, A. Genetic Engineering of Snake Toxins: The functional site of Erabutoxin a, as delineated by site-directed mutagenesis, includes variant residues. *J. Biol. Chem.* 1995, 270, 9362–9369. [CrossRef] [PubMed]

121. Teixeira-Clerc, F.; Ménez, A.; Kessler, P. How Do Short Neurotoxins Bind to a Muscular-Type Nicotinic Acetylcholine Receptor? *J. Biol. Chem.* 2002, 277, 25741–25747. [CrossRef] [PubMed]

122. Ackermann, E.J.; Taylor, P. Nonidentity of the α-Neurotoxin Binding Sites on the Nicotinic Acetylcholine Receptor Revealed by Modification in α-Neurotoxin and Receptor Structures. *Biochemistry* 1997, 36, 12836–12844. [CrossRef] [PubMed]

123. Osaka, H.; Malany, S.; Kanter, J.R.; Sine, S.M.; Taylor, P. Subunit Interface Selectivity of the Alpha-Neurotoxins for the Nicotinic Acetylcholine Receptor. *J. Biol. Chem.* 1999, 274, 9581–9586. [CrossRef]
124. Roy, A.; Zhou, X.; Chong, M.Z.; D’hoedt, D.; Foo, C.S.; Rajagopalan, N.; Nirthanan, S.; Bertrand, D.; Sivaraman, J.; Kini, R.M. Structural and Functional Characterization of a Novel Homodimeric Three-Finger Neurotoxin from the Venom of Ophiophagus Hannah (King Cobra). J. Biol. Chem. 2010, 285, 8302–8315. [CrossRef] [PubMed]

125. Betzel, C.; Lange, G.; Pal, G.P.; Wilson, K.S.; Maelicke, A.; Saenger, W. The Refined Crystal Structure of Alpha-Cobratoxin from Naja Naja Siamesis at 2.4-A Resolution. J. Biol. Chem. 1991, 266, 21530–21536. [CrossRef]

126. Chatrath, S.T.; Chapeaurouge, A.; Lin, Q.; Lim, T.K.; Dunstan, N.; Mirtschin, P.; Kumar, P.P.; Kini, R.M. Identification of Novel Proteins from the Venom of a Cryptic Snake Drysdalia Coronoides by a Combined Transcriptomics and Proteomics Approach. J. Proteome Res. 2011, 10, 739–790. [CrossRef] [PubMed]

127. Pillet, L.; Trémeau, O.; Ducancel, F.; Drevet, P.; Zinn-Justin, S.; Pinkasfeld, S.; Boulain, J.C.; Ménez, A. Genetic Engineering of Snake Toxins. Role of Invariant Residues in the Structural and Functional Properties of a Curaremimetic Toxin, as Probed by Site-Directed Mutagenesis. J. Biol. Chem. 1993, 268, 909–916. [CrossRef]

128. Dewan, J.C.; Grant, G.A.; Sacchettini, J.C. Crystal Structure of Kappa-Bungarotoxin at 2.3-A Resolution. Biochemistry 1994, 33, 13147–13154. [CrossRef]

129. Chiappinelli, V.A.; Weaver, W.R.; McLane, K.E.; Conti-Fine, B.M.; Fior dalisi, J.J.; Grant, G.A. Binding of Native κ-Nicotinotoxins and Site-Directed Mutants to Nicotinic Acetylcholine Receptors. Biochemistry 1996, 34, 1243–1256. [CrossRef]

130. Mordvintsev, D.Y.; Polyak, Y.L.; Rodionov, D.J.; Jakubik, J.; Dolezal, V.; Karlsson, E.; Tsetlin, V.I.; Ut kin, Y.N. Weak Toxin WTX from Naja Kaouthia Cobra Venom Interacts with Both Nicotinic and Muscarinic Acetylcholine Receptors. FEBS J. 2009, 276, 5065–5075. [CrossRef]

131. Lyukmanova, E.N.; Shulepmko, M.A.; Shenkarev, Z.O.; Kasheverov, I.E.; Chugunov, A.O.; Kulbatskii, D.S.; Myshkin, M.Y.; Ut kin, Y.N.; Efremov, R.G.; Tsetlin, V.I.; et al. Central Loop of Non-Conventional Toxin WTX from Naja Kaouthia Is Important for Interaction with Nicotinic Acetylcholine Receptors. Toxicon 2016, 119, 274–279. [CrossRef] [PubMed]

132. Pawlak, J.; Mackessy, S.P.; Fry, B.G.; Bhatia, M.; Mourier, G.; Frucht-Gaillard, C.; Servent, D.; Ménez, R.; Stura, E.; Ménez, A.; et al. Denmotoxin, a Three-Finger Toxin from the Colubrid Snake Boiga Dendrophila (Mangrove Catsnake) with Bird-Specific Activity. J. Biol. Chem. 2006, 281, 29030–29041. [CrossRef] [PubMed]

133. Kim, H.S.; Tamiya, N. Amino Acid Sequences of Two Novel Long-Chain Neurotoxins from the Venom of the Sea Snake Laticauda Colubrina. Biochem. J. 1982, 207, 215–223. [CrossRef] [PubMed]

134. Chang, L.-S.; Liou, J.-C.; Lin, S.-R.; Huang, H.-B. Purification and Characterization of a Neurotoxin from the Venom of Ophiophagus Hannah (King Cobra). Biochim. Biophys. Acta. 2002, 294, 574–578. [CrossRef]

135. Hassan-Puttaswamy, V.; Adams, D.J.; Kini, R.M. A Distinct Functional Site in Ω-Neurotoxins: Novel Antagonists of Nicotinic Acetylcholine Receptors from Snake Venom. ACS Chem. Biol. 2015, 10, 2805–2815. [CrossRef]

136. Moll es, B.E.; Rezai, P.; Kline, E.F.; Mc Ardl e, J.J.; Sin e, S.M.; Tay lor, P. Identification of Residues at the α and γ Subunit Interfaces Mediating Species Selectivity of Waglerin-1 for Nicotinic Acetylcholine Receptors. J. Biol. Chem. 2002, 277, 5433–5440. [CrossRef] [PubMed]

137. Ye, J.-H.; Mc Ardle, J.J. Waglerin-1 Modulates γ-Aminobutyric Acid Activated Current of Murine Hypothalamic Neurons. J. Pharmacol. Exp. Ther. 1997, 282, 74.

138. Sellin, L.C.; Mattila, K.; Annila, A.; Schmidt, J.J.; Mc Ardle, J.J.; Hyvönen, M.; Rantal a, T.T.; Kivistö, T. Conformational Analysis of a Toxic Peptide from Trimeresurus Wagleri Which Blocks the Nicotinic Acetylcholine Receptor. Bio ph ys. J. 1996, 70, 3–13. [CrossRef]

139. Lyukmanova, E.N.; Shenkarev, Z.O.; Shulepmko, M.A.; Paramonov, A.S.; Chugunov, A.O.; Janickova, H.; Dolejsi, E.; Dolezal, V.; Ut kin, Y.N.; Tsetlin, V.I.; et al. Structural Insight into Specificity of Interactions between Nonconventional Three-Finger Weak Toxin from Naja Kaouthia (WTX) and Muscarinic Acetylcholine Receptors. J. Biol. Chem. 2015, 290, 23616–23630. [CrossRef]

140. Mc Ardle, J.J.; Lentz, T.L.; Witzemann, V.; Schwarz, H.; Weinstein, S.A.; Schmidt, J.J. Waglerin-1 Selectively Blocks the Epsilon Form of the Muscle Nicotinic Acetylcholine Receptor. J. Pharmacol. Exp. Ther. 1999, 289, 543.

141. Liang, S.; Zhang, D.; Pan, X.; Chen, Q.; Zhou, P. Properties and Amino Acid Sequence of Huwentoxin-I, a Neurotoxin Purified from the Venom of the Chinese Bird Spider Selenocosmia Huwena. Toxicon 1993, 31, 969–978. [CrossRef]

142. Zhou, P.; Xie, X.; Li, M.; Yang, D.; Xie, Z.-P.; Zong, X.; Liang, S. Blockade of Neuromuscular Transmission by Huwentoxin-I, Purified from the Venom of the Chinese Bird Spider Selenocosmia Huwena. Toxicon 1997, 35, 39–45. [CrossRef]

143. Liang, S.-P.; Chen, X.-D.; Shu, Q.; Zhang, Y.; Peng, K. The Presynaptic Activity of Huwentoxin-I, a Neurotoxin from the Venom of the Chinese Bird Spider Selenocosmia Huwena. Toxicon 2000, 38, 1237–1246. [CrossRef]

144. Peng, K.; Chen, X.D.; Liang, S.P. The Effect of Huwentoxin-I on Ca(2+) Channels in Differentiated NG108-15 Cells, a Patch-Clamp Study. Toxicon 2001, 39, 491–498. [CrossRef]

145. Wang, M.; Guan, X.; Liang, S. The Cross Channel Activities of Spider Neurotoxin Huwentoxin-I on Rat Dorsal Root Ganglion Neurons. Biochem. Biophys. Res. Comm. 2007, 357, 579–583. [CrossRef] [PubMed]

146. Granja, R.; Fernández-Fernández, J.; Izaguirre, V.; González-García, C.; Ceña, V. ω-Agatoxin IVA Blocks Nicotinic Receptor Channels in Bovine Chromaffin Cells. FEBS Lett. 1995, 362, 15–18. [CrossRef]

147. Wang, J.; Irnaten, M.; Mendelowitz, D. Agatoxin-IVA-Sensitive Calcium Channels Mediate the Presynaptic and Postsynaptic Nicotinic Activation of Cardiac Vagal Neurons. J. Neurophysiol. 2001, 85, 164–168. [CrossRef]

148. Adams, M.E. Agatoxins: Ion Channel Specific Toxins from the American Funnel Web Spider, Agelenopsis Aperta. Toxicon 2004, 43, 509–525. [CrossRef]
149. Windley, M.J.; Vetter, I.; Lewis, R.J.; Nicholson, G.M. Lethal Effects of an Insecticidal Spider Venom Peptide Involve Positive Allosteric Modulation of Insect Nicotinic Acetylcholine Receptors. *Neuropharmacology* **2017**, *127*, 224–242. [CrossRef]

150. Bergeron, Z.L.; Bingham, J.-P. Scorpion Toxins Specific for Potassium (K+) Channels: A Historical Overview of Peptide Bioengineering. *Toxins* **2012**, *4*, 1082–1119. [CrossRef]

151. Gurevitz, M.; Gordon, D.; Barzilai, M.G.; Kahn, R.; Cohen, L.; Moran, Y.; Zilberberg, N.; Froy, O.; Altman-Gueta, H.; Turkov, M.; et al. Molecular Description of Scorpion Toxin Interaction with Voltage-Gated Sodium Channels. In *Scorpion Venoms*; Gopalakrishnakone, P., Possani, L.D., Schwartz, E., Rodríguez de la Vega, R.C., Eds.; Springer: Dordrecht, The Netherlands, 2015; pp. 471–491. ISBN 978-94-007-6404-0.

152. Quintero-Hernández, V.; Jiménez-Vargas, J.M.; Gurrola, G.B.; Valdivia, H.H.; Possani, L.D. Scorpion Venom Components That Affect Ion-Channels Function. *Toxicon* **2013**, *76*, 328–342. [CrossRef] [PubMed]

153. Kasheverov, I.E.; Oparin, P.B.; Zhmak, M.N.; Egorova, N.S.; Ivanov, I.A.; Gigolaev, A.M.; Nekrasova, O.V.; Serebryakova, M.V.; Kudryavtsev, D.S.; Prokopec, N.A.; et al. Scorpion Toxins Interact with Nicotinic Acetylcholine Receptors. *FEBS Lett.* **2019**, *593*, 2779–2789. [CrossRef] [PubMed]

154. Mouhat, S.; Visan, V.; Ananthakrishnan, S.; Wulff, H.; Andreotti, N.; Grissmer, S.; Darbon, H.; De Waard, M.; Sabatier, J.-M. K+ Channel Types Targeted by Synthetic OSK1, a Toxin from Orthochirus Scrobiculosus Scorpion Venom. *Biochem. J.* **2004**, *385*, 95–104. [CrossRef] [PubMed]