

## Two Potent $\alpha 3/5$ Conotoxins from Piscivorous *Conus achatinus*

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**Abstract** Every cone snail produces a mixture of different conotoxins and secretes them to immobilize their prey and predators.  $\alpha 3/5$  Conotoxins, isolated from fish-hunting cone snails, target muscle nicotinic acetylcholine receptors. The structure and function of  $\alpha 3/5$  conotoxin from the piscivorous *Conus achatinus* have not been studied. We synthesized two pentadecamer peptides, Ac1.1a and Ac1.1b, with appropriate disulfide bonding, based on cDNA sequences of  $\alpha 3/5$  conotoxins from *C. achatinus*. Ac1.1a and Ac1.1b differ by only one amino acid residue. They have similar potency on blocking recombinant mouse muscle acetylcholine receptor expressed in *Xenopus laevis* oocytes, with IC<sub>50</sub> values of 36 nM and 26 nM, respectively. For Ac1.1b, deletion of the first three N-terminal amino acids did not change its activity, indicating that the N-terminus is not involved in the interaction with its receptor. Furthermore, our experiments indicate that both toxins strongly prefer the  $\alpha_1$ - $\delta$  subunit interface instead of the  $\alpha_1$ - $\gamma$  binding site on the mouse muscle nicotinic acetylcholine receptor. These peptides provide additional tools for the study of the structure and function of nicotinic receptor.

**Keywords** cone snail;  $\alpha 3/5$  conotoxin; disulfide bond; nicotinic acetylcholine receptor

Cone snails are marine gastropod mollusks that eat fish, worms, or other mollusks. These predatory snails are not notable for either speed or mechanical weaponry. However, they have developed several types of small peptide toxins, conotoxins, that act rapidly on different receptors and ion channels expressed within the nervous system. There are approximately 500 different venomous *Conus*, and each species is estimated to generate between 50 and 200 unique conotoxins, only a small fraction of which have been characterized in detail [1]. The majority of biologically active conotoxins that have been described are small peptides of 10–40 amino acids, often with multiple disulfide bonds.

It is known that most conotoxin genes encode a precursor comprised of a signal peptide, pro-peptide, and the mature conotoxin at the C-terminus. Conotoxins are

grouped into several superfamilies based on the sequence of the signal peptide of their precursors. To date, the A-, O-, M-, P-, I-, S-, and T-superfamilies have been reported. Each superfamily contains conotoxins with different arrangements of cysteine residues and modes of action [2]. The mechanisms that lead to conservation of signal peptides, particularly as compared to the sequence hyperdivergence of mature conotoxins, remain a subject for further study.

The A-superfamily is relatively well characterized compared to other conotoxin superfamilies and is comprised of three distinct pharmacological families,  $\alpha$ -,  $\alpha A$ - and  $\kappa A$ -conotoxins [3].  $\alpha$ -Conotoxins, widely distributed in the venom of most *Conus* species, are competitive antagonists of the nicotinic acetylcholine receptor (nAChR). Most of these  $\alpha$ -conotoxins have four cysteine residues in an arrangement of CCX<sub>3</sub>CX<sub>5</sub>C ( $\alpha 3/5$  subfamily), CCX<sub>4</sub>CX<sub>3</sub>C ( $\alpha 4/3$  subfamily), or CCX<sub>4</sub>CX<sub>7</sub>C ( $\alpha 4/7$  subfamily), where X represents any amino acid. A conserved proline is present in the first Cys loop in almost all  $\alpha$ -conotoxins described to date [4].

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The targets of  $\alpha$ -conotoxins, nAChRs, form cation channels that open in response to acetylcholine, a major neurotransmitter at certain synaptic contacts within the nervous system, including the neuromuscular junction [5]. The pentameric nAChRs can be divided into muscle and neuronal types. Muscle nAChRs contain two  $\alpha_1$  subunits along with  $\beta_1$ ,  $\gamma$  and  $\delta$  subunits. In contrast, a distinct and diverse set of  $\alpha$  and  $\beta$  subunits comprise neuronal nAChRs.  $\alpha$ -Conotoxins are very selective towards different subclasses of nAChR and have become invaluable tools for nAChR study [6].

$\alpha$ 3/5 Conotoxins, found only in fish-hunting cone snails, selectively block muscle nAChRs and disrupt neuromuscular transmission to paralyze prey [7]. In muscle nAChRs there are two ligand-binding sites, located at the interfaces between  $\alpha$  and  $\gamma$  subunits or  $\alpha$  and  $\delta$  subunits.  $\alpha$ 3/5 Conotoxins discriminate between these two ligand-binding sites, displaying binding affinities for these sites that differ by three to four orders of magnitude. For example,  $\alpha$ -conotoxin MI has an approximate  $10^4$  preference for the binding site at the  $\alpha_1$ - $\delta$  interface over the site at the  $\alpha_1$ - $\gamma$  interface of mammalian nAChR [4]. In contrast, the snake toxin,  $\alpha$ -bungarotoxin (Bgtx), has similar affinities for the two ligand-binding sites of muscle nAChRs and inhibits the homopentameric  $\alpha_7$  subtype of neuronal nAChR [8].

*Conus achatinus* is a fish-hunting cone snail indigenous to the Pacific and Indian Oceans. To date, no  $\alpha$ 3/5 conotoxin has been purified from this species. We have identified two  $\alpha$ 3/5 conotoxin cDNAs, Ac1.1a and Ac1.1b (GenBank Accession No. DQ359138 and DQ359139) from this *Conus* species. Here, we describe the chemical synthesis and biological activities of these two novel  $\alpha$ 3/5 conotoxins.

## Materials and Methods

### Materials

Zorbax 300SB-C18 semipreparative column was purchased from Agilent Technologies (Santa Clara, USA), and trifluoroacetic acid (TFA) and acetonitrile for HPLC were from Merck (Darmstadt, Germany). Other reagents were of analytical grade.

### Peptide synthesis

The linear peptides were synthesized on an ABI 431A peptide synthesizer (ABI, Foster City, USA) using Rink amide resin and standard Fmoc [N-(9-fluorenyl)methoxy-

carbonyl] chemistry. Side-chain protecting groups of non-Cys residues were t-butyl (Ser) and trityl (Asn). Orthogonal protection was used on cysteines: Cys4 and Cys9 were protected as the stable Cys (S-acetamidomethyl); Cys5 and Cys15 were protected as the acid-labile Cys (S-trityl). Linear peptides were cleaved from the resin by treatment with TFA/H<sub>2</sub>O/ethanedithiol/phenol/thioanisole (90:5:2.5:7.5:5 by volume), the Cys (S-trityl) and other residue side chains were deprotected at the same time. Released peptides were precipitated and washed several times with cold methyl-t-butyl ether (MTBE), then purified on an RP-HPLC C18 column (9.4 mm $\times$ 250 mm).

### Two-step folding of synthesized $\alpha$ -conotoxins

The disulfide bond between Cys5 and Cys15, deprotected during cleavage of the peptide from resin, was formed by air oxidation. The linear peptide (5 mM in 0.1% TFA) was added into 100 volumes of reaction buffer (50 mM NH<sub>4</sub>HCO<sub>3</sub>, pH 8.0, 1 mM reduced and 0.1 mM oxidized glutathione) and stirred for 8 h at room temperature. The single disulfide bonded products were purified on the RP-HPLC C18 column (9.4 $\times$ 250 mm). Then the S-acetamidomethyl group was removed from Cys4 and Cys9 in 0.02 mg/ml iodine, 4% TFA and 10% acetonitrile. The disulfide bond between these two cysteines was formed simultaneously. The reaction was quenched by adding ascorbic acid before purification of the final products on the C18 column. All the purifications were carried out with the same elution gradient, 15%–26% buffer B in 11 min. Buffer A was 0.1% TFA and buffer B was 0.1% TFA in acetonitrile.

### Toxicity assay

The synthesized and folded  $\alpha$ 3/5 conotoxin Ac1.1b was dissolved in 0.9% NaCl for the lethal dosage measurement. Various amounts of Ac1.1b in 200  $\mu$ l volume were injected intravenously into 28–30 g Kunming male mice. Survival times were recorded and used to calculate the LD<sub>50</sub> value as reported previously [9].

### RNA preparation and oocyte injection

RNA for mouse muscle nAChR subunits and rat neuronal nAChR subunits was transcribed using mMESSAGE mMACHINE (ABI, Foster City, USA) with either SP6 or T7 polymerases and precipitated with lithium chloride. Oocytes were harvested from *Xenopus laevis* and dissociated in solution of 1.5 mg/ml collagenase II at room temperature for 1 h. Defolliculated oocytes were injected with 50 nl of cRNA using a subunit ratio of 2- $\alpha$ :3- $\beta$  subunits for neuronal receptors, or 2- $\alpha$ :1- $\delta$ :1- $\gamma$ :1- $\beta_1$  for muscle

receptors, at a total concentration of 0.125  $\mu\text{g}/\mu\text{l}$  for each subunit [10,11]. Oocytes were incubated at 15 °C in ND96 buffer with 1 mM glucose-6-phosphate to increase oocyte survival for up to 7 d after injection [10,12].

### Electrophysiological recordings

nAChR currents were measured using a two-electrode voltage clamp on an OC-725C amplifier (Warner Instruments, Hamden, USA). Oocytes were placed in a Warner RC-3Z recording chamber and attached with an OC-725 bath clamp. Electrodes with a resistance between 0.05 and 0.2 M $\Omega$  were filled with 3 M KCl. For IC<sub>50</sub> experiments, oocytes were clamped at -60 mV and the chamber perfused with OR2 at 5 ml/min by gravity flow controlled by a Warner BPS-8 controller [13]. A controlled dose of 10  $\mu\text{M}$  ACh for muscle constructs and 100  $\mu\text{M}$  ACh for neuronal constructs was perfused onto oocytes for 5 s every 2 min to obtain baseline activity. Solutions with different conotoxin concentrations were each perfused onto oocytes for 15 s and the oocytes were subsequently incubated without perfusion for 5 min. ACh was reapplied to assess the amount of inhibition and additional ACh responses were recorded to observe oocyte recovery from toxin exposure [14]. At least five different conotoxin concentrations were tested for each derivative. All conotoxin solutions contained 0.2 mg/ml bovine serum albumin to prevent toxin aggregation.

## Results and Discussion

### Primary structure of Ac1.1a and Ac1.1b

In previous work from our laboratory, cDNAs of two  $\alpha 3/5$  conotoxins, Ac1.1a and Ac1.1b, were cloned from

the fish-hunting cone snail *C. achatinus* (GenBank accession No. DQ359138 and DQ359139). These two cDNAs encode precursors that each contain an N-terminal signal peptide of 21 residues, an intervening pro-peptide of 26 residues, a C-terminal mature toxin region of 15 amino acids, and two additional residues GK that are removed during the maturation of the toxins and result in the amidation of the C-terminus of toxins [15] (**Table 1**). The N-terminal signal sequence (MGMRMMFTVFLLVV LATTVVVS) is a defining feature of all A-superfamily conotoxins [16]. The Cys pattern (CCX<sub>3</sub>CX<sub>5</sub>C) of Ac1.1a and Ac1.1b defines them as  $\alpha 3/5$  conotoxins. Their sequences are homologous with other  $\alpha 3/5$  conotoxins (**Table 1**). In particular, the Pro and Ala residues in the first cysteine loop, as well as the Gly and Tyr/Phe residues at the first and fourth positions in the second cysteine loop, are conserved in every  $\alpha 3/5$  conotoxin characterized so far [17–19].

There is only one amino acid difference, Gln or Ser at position 14, between Ac1.1a and Ac1.1b. Polymorphisms are quite common among conotoxins, such as  $\alpha 4/7$  conotoxin AuIA and AuIC that differ by only one residue [20]. This most likely arises from hypermutation of conotoxin genes after duplication, which is believed to be the mechanism by which cone snails generate hypervariable toxins [21]. The mutation rate in conotoxin genes has been calculated to be 5–10 times higher than normal gene evolution [22,23]. However, the mechanism of conotoxin gene hypermutation has not been well elucidated.

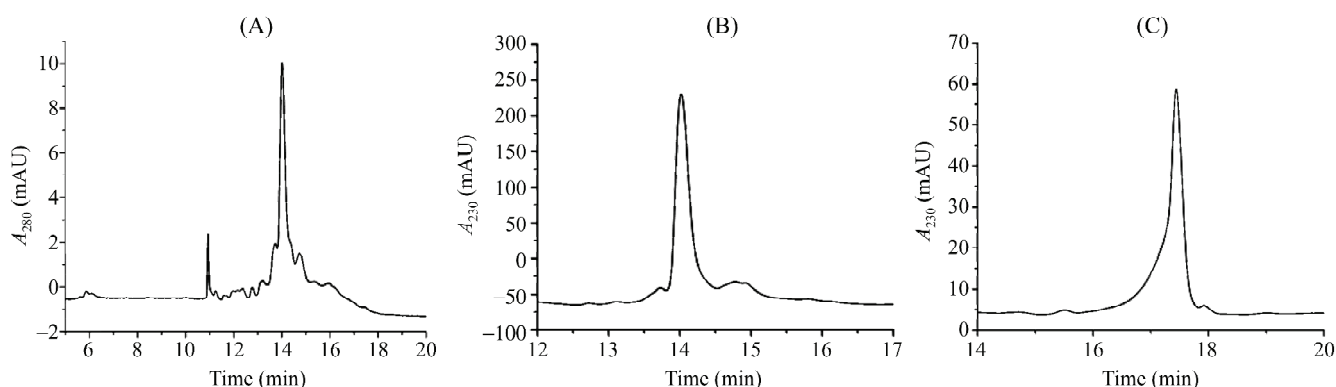
### Peptide synthesis and oxidation

We chemically synthesized Ac1.1a and Ac1.1b conotoxins in order to study their functional properties. Based on high sequence homology, the disulfide connectivity of these two toxins is assumed to be the same as

**Table 1** Precursor sequences of a selection of  $\alpha 3/5$  conotoxins

Conotoxin	Signal peptide	Pro-region	Mature peptide	Ref.
GI	MGMRMMFTVFLLVV LATTVVVS	<u>FPSERASDGRDDTAKDEGSDMEKLVEKKECCNPACGRHYS</u> CGR		[3]
GIB	MGMRMMFTVFLLVV LATTVVVS	<u>FPSERASDGRDDTAKDEGSDMEKLVEKKECCNPACGRHYS</u> CKGGR		[3]
SI	MGMRMMFTVFLLVV LATTVVVS	<u>FPSDRASDGRDDEAKDERSDMHESDRKE</u> ICCNPA <b>C</b> GPKYSCGR		[4]
MI	MGMRMMFTVFLLVV LTTTVVS	<u>FPSDRASDGRDDEAKDERSDMYESKRD</u> GR <b>C</b> CHPA <b>C</b> GGKNSCGR		[17]
CnIA	MGMRMMFTVFLLVV LTTTVVS	<u>FPSDSASDVRDDEAKDERSDMYKSKRNGR</u> CC <b>H</b> PA <b>C</b> GGKYSCGR		[24]
Ac1.1a	MGMRMMFTLFLLVV LTTTVVS	<u>YPSDSASDGRDDEAKDERSDMYELKRNGR</u> CC <b>H</b> PA <b>C</b> GGKHFNCGR		†
Ac1.1b	MGMRMMFTLFLLVV LTTTVVS	<u>FPSDSASDGRDDEAKDERSDMYKSKRNGR</u> CC <b>H</b> PA <b>C</b> GGKHFSCGR		†

† The sequences of Ac1.1a and Ac1.1b are from Genbank accession No. DQ359138 and DQ359139, respectively. The N-terminal peptides of 21 residues are signal peptides, which are conserved in all the A-superfamily conotoxins. The pro-peptides are underlined. The mature peptides are shaded differently to show different variations. Cys residues are in bold.



**Fig. 1** HPLC profiles of  $\alpha 3/5$  conotoxin Ac1.1b from *Conus achatinus* at different folding status

(A) The synthesized linear peptide of Ac1.1b was eluted at 14.3 min. (B) Ac1.1b with the first disulfide bond Cys5–Cys15 formed. The elution time was 14.0 min. (C) Purification of the bicyclic Ac1.1b on HPLC. The elution time was 17.2 min. All elution processes were carried out using the following protocol: 0–5 min, 0–15% buffer B; 5–16 min, 15%–26% buffer B; 16–26 min, 26%–100% buffer B. Buffer B was 0.1% TFA in acetonitrile.  $A_{280}$  refers to the absorbance at 280 nm in milli absorbance unit.

that of all other  $\alpha 3/5$  conotoxins identified: C<sub>1</sub>–C<sub>3</sub>, C<sub>2</sub>–C<sub>4</sub>. After obtaining linear peptides, the disulfide bond between Cys5 and Cys15 was formed first, followed by the Cys4–Cys9 disulfide bond. The final folded peptides were purified on HPLC. Successful peptide synthesis and the formation of each disulfide bond were confirmed by mass spectrometry (data not shown).

Notably, the folded toxins were more hydrophobic than their corresponding linear and monocyclic peptides, with elution times on HPLC of 17.2 min for the folded Ac1.1b toxin and 14.3 min for the linear peptide (**Fig. 1**). From what is known of the structures of other  $\alpha 3/5$  conotoxins, this might result from clustering of hydrophobic residues, Pro7, Ala8, Gly10 and Tyr13, as a consequence of the disulfide bridge between Cys4 and Cys9.

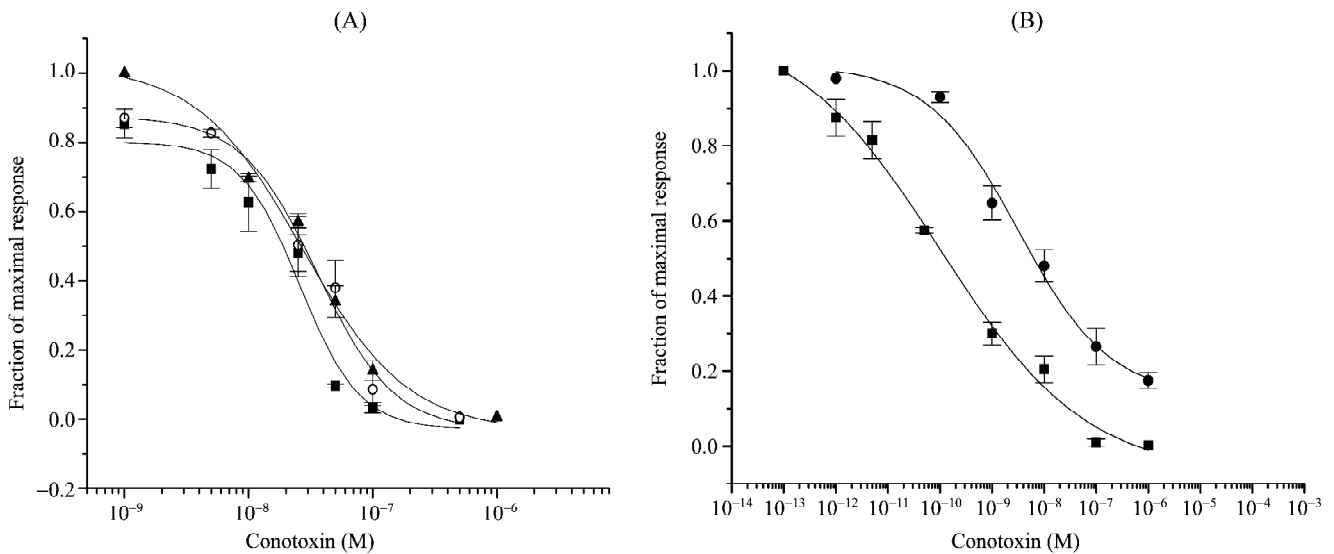
The elution peaks of the final folded toxins are also asymmetric (**Fig. 1**). These asymmetries could not be improved even when the latter phase of the peak was re-run on HPLC. The molecular weights are the same for different fractions of the peak, suggesting that these toxins can reside in two conformations and transition between them in solution. Similar results have been reported for other  $\alpha 3/5$  conotoxins [24,25]. The reported structures of  $\alpha 3/5$  conotoxins represent the major conformation [26], whereas the minor conformation has been analyzed only for conotoxin GI [28]. These two conformations differ in the regions containing the second cysteine loop and peptide termini [28]. The two conformations are interconvertible in solution [28], but lack of detailed structural information of these toxins in complex with nAChR makes it difficult to determine which is favorable for the activity.

## Functional studies

To confirm that the synthesized toxins are active, we injected Ac1.1b intravenously into Kunming mice. The typical symptoms of flaccid paralysis followed by death were observed. The LD<sub>50</sub> value of Ac1.1b is 38±9 µg/kg.

The activities of Ac1.1a and Ac1.1b were then tested on neuronal and muscle nAChRs. Neuronal and muscle nAChRs were expressed in *X. laevis* oocytes and baseline currents were measured in response to 100 µM or 10 µM Ach application. We delivered conotoxins at a variety of concentrations and determined the degree of inhibition for each concentration. The fraction of the maximal response remaining after conotoxin application was plotted against conotoxin concentration, and the resulting data were fitted with the Hill equation functions to derive IC<sub>50</sub> values [**Fig. 2(A)** and **Table 2**]. Ac1.1a and Ac1.1b were characterized by IC<sub>50</sub> values in the nanomolar range (27–36 nM) for muscle nAChR ( $\alpha_1$ )<sub>2</sub> $\delta\gamma\beta_1$ . In comparison, Bgtx is characterized by an IC<sub>50</sub> of 2 nM on muscle nAChRs [11]. Ac1.1a and Ac1.1b did not appear to inhibit  $\alpha_3\beta_2$  or  $\alpha_3\beta_4$  neuronal nAChR under the conditions used. This is similar to Bgtx, which also fails to bind to these neuronal receptors (**Table 2**).

The sequences of the  $\alpha 3/5$  conotoxins from different *Conus* species are homologous, except for the N-terminal extension prior to the first Cys residue (**Table 1**). To clarify the effect of the N-terminal sequence on the activity of  $\alpha 3/5$  conotoxins, we deleted the N-terminus of Ac1.1b. Ac1.1b- $\Delta$ N was synthesized and folded in the same way as the other two toxins. Ac1.1b- $\Delta$ N showed the same



**Fig. 2** Inhibitory effects of conotoxins Ac1.1a, Ac1.1b and Ac1.1b-ΔN on mouse muscle nicotinic acetylcholine receptor (nAChR) expressed in *Xenopus laevis* oocytes

(A) Inhibition curves of Ac1.1a (○;  $n=2-4$ ), Ac1.1b (■;  $n=2-4$ ) and Ac1.1b-ΔN (▲;  $n=2-4$ ) on the complete  $(\alpha_1)_2\beta\delta\gamma$  nAChR. (B) Inhibition curve of Ac1.1a (●;  $n=2-4$ ) and Ac1.1b (■;  $n=2-4$ ) on mouse muscle nAChR with only  $\alpha\beta\delta$  subunits. Various concentrations of peptides were applied and the fraction of the maximal response was plotted. Data points were fitted with the Hill equation. Error bars indicate SD.

**Table 2**  $IC_{50}$  values for snake toxin  $\alpha$ -bungarotoxin (Bgtx) and conotoxins Ac1.1a, Ac1.1b and Ac1.1b-ΔN on different nicotinic acetylcholine receptor (nAChR) constructs

nAChR construct	$IC_{50}$ (nM)			
	Bgtx	Ac1.1a	Ac1.1b	Ac1.1b-ΔN
$\alpha_1\delta\gamma\beta_1$	1.9±0.3	35.9±7.7	25.8±5.9	27.0±7.1
$\alpha_2\beta_2$	>10000 †	>5000	>5000	>3000
$\alpha_3\beta_4$	>10000 †	>50000	>50000	>5000
$\alpha_1\delta\beta_1$	ND	3.2±2.2	0.101±0.064	ND
$\alpha_1\gamma\beta_1$	ND	>50000	>50000	ND

† Data from Levandoski *et al.* [10]. ND, no data.

activity of nAChR current inhibition as the full-length Ac1.1b (Table 2). This strongly indicates that the N-terminal extension of the  $\alpha_3/5$  conotoxins is not crucial for the nAChR binding activity. Similar results have been obtained from other  $\alpha_3/5$  conotoxin studies. For example, removing the N-terminal Glu from conotoxin GI or the N-terminal Ile of conotoxin SI does not significantly alter their biological activity [26,27].

Among the  $\alpha_3/5$  conotoxins characterized so far, conotoxins MI and GI have also been tested against recombinant mouse nAChR expressed in *Xenopus* oocytes [29–31], and therefore could be easily compared with our

results for Ac1.1a and Ac1.1b (Table 3). All four  $\alpha_3/5$  conotoxins have similar activities, with 20–40 nM toxin concentrations giving half maximal inhibition (although one study found the  $IC_{50}$  of conotoxin MI to be 0.4 nM) (Table 3). Consistent with their similar activities, the four  $\alpha_3/5$  conotoxins share very high sequence homology except for the first three N-terminal residues prior to the Cys4, which appears not to be essential for function. In particular, the conserved Pro and Phe/Tyr residues at positions 7 and 13, respectively, in the Ac1.1a sequence have been shown to be critical for the hydrophobic interaction between the  $\delta$  subunit of nAChR and conotoxin MI [30,32]. These

**Table 3** Activities of the  $\alpha$ 3/5 conotoxins assayed on mouse muscle nicotinic acetylcholine receptor expressed in *Xenopus laevis* oocytes

Conotoxin	Sequence	IC <sub>50</sub> (nM)	Ref.
MI	GR <b>CC</b> HPACGK <b>NY</b> SC*	0.4±0.17	[29]
		12.0±1.3	[30]
GI	E <b>CC</b> NPACGRH <b>Y</b> SC*	20.3±1.0	[25]
Ac1.1a	NGR <b>CC</b> HPACGK <b>HF</b> QC*	35.9±7.7	This work
Ac1.1b	NGR <b>CC</b> HPACGK <b>HF</b> SC*	25.8±5.9	This work
Ac1.1b-ΔN	<b>CC</b> HPACGK <b>HF</b> SC*	27.0±7.1	This work

\* Indicates the C-terminal amidation. The cysteine residues are in bold, the conserved residues are shaded.

two residues are apparently conserved in all the  $\alpha$ 3/5 conotoxins. However, residues that are less well-conserved, such as residues 6, 12, and 14 (numbers based on Ac1.1 peptides) have little effect on toxin binding to nAChR.

Sequence variations within different regions of  $\alpha$ 3/5 conotoxins strongly suggest that binding to nAChR is primarily mediated by two conserved Cys loops, and the N-terminus resides outside of the interaction site. This hypothesis was further supported by a model derived from photoactivated crosslinking of an  $\alpha$ 3/5 conotoxin GI analog to a muscle nAChR [33]. Evidence from crystal structures obtained for conotoxin bound to soluble ACh binding protein, homologous to the extracellular domain of nAChR, indicates that  $\alpha$ 4/3 and  $\alpha$ 4/7 conotoxins bind in a similar orientation to nAChR [34,35]. As the structure-function relationship studies have indicated that the functionally important residues are located in the two Cys loops for all the  $\alpha$ -conotoxins [36], it is highly possible that  $\alpha$ 3/5 conotoxins bind with nAChR in the same way, although the high resolution structure of the toxin-receptor complex is needed to clarify this point.

We are also interested in the site specificity of  $\alpha$ 3/5 conotoxins. In muscle nAChR, there are two potential binding sites for ACh and conotoxins: between the  $\alpha_1$  and  $\delta$  subunits; and between the  $\alpha_1$  and  $\gamma$  subunits. To test if either Ac1.1a or Ac1.1b conotoxins prefer one of these binding sites over the other, we expressed the muscle nAChR lacking either  $\delta$  or  $\gamma$  subunits. Ac1.1a and Ac1.1b blocked the ACh evoked current through receptors lacking the  $\gamma$  subunit (IC<sub>50</sub> as low as 3.2 nM and 101 pM, respectively) [Fig. 2(B)]. When the  $\delta$  subunit was absent, they failed to inhibit ACh evoked currents (Table 2). These data indicate that Ac1.1a and Ac1.1b might block the channel by preferentially binding to the  $\alpha_1$ - $\delta$  binding site instead of the  $\alpha_1$ - $\gamma$  site. This is similar to other  $\alpha$ 3/5 conotoxins [4,29]. The high selectivity of  $\alpha$ 3/5 conotoxins

makes them unique and invaluable tools for the study of muscle nAChR. High resolution structure of the toxin-receptor complex could elucidate the origin of their site selectivity.

In summary, two  $\alpha$ 3/5 conotoxins from fish-hunting *C. achatinus*, Ac1.1a and Ac1.1b, were found to be potent antagonists of recombinant mouse muscle nAChR. They might be used by the snail to paralyze prey. These two toxins share high sequence homology, similar functional activity, and site selectivity as other  $\alpha$ 3/5 conotoxins. The most variable sequence of these toxins, the N-terminal extension, does not affect activity. Thus conserved regions of  $\alpha$ 3/5 conotoxins likely impart their common activity. This will improve the understanding of the structure and function of  $\alpha$ 3/5 conotoxins.

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