A New α-Conotoxin Which Targets α3β2 Nicotinic Acetylcholine Receptors*

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G. Edward Cartier‡, Doju Yoshikami‡, William R. Gray‡, Siqin Luo‡, Baldomero M. Olivera‡, and J. Michael McIntosh§

From the Departments of Biology and Psychiatry, University of Utah, Salt Lake City, Utah 84112

We have isolated a 16-amino acid peptide from the venom of the marine snail Conus magus which potently blocks nicotinic acetylcholine receptors (nAChRs) composed of α3β2 subunits. This peptide, named α-conotoxin MIW, was identified by electrophysiologically screening venom fractions against cloned nicotinic receptors expressed in Xenopus oocytes. The peptide’s structure, which has been confirmed by mass spectrometry and total chemical synthesis, differs significantly from those of all previously isolated α-conotoxins. Disulfide bridging, however, is conserved. The toxin blocks the response to acetylcholine in oocytes expressing α3/β2 nAChRs with an IC50 of 0.5 nM and is 2-4 orders of magnitude less potent on other nAChR subunit combinations. We have recently reported the isolation and characterization of α-conotoxin Im1, which selectively targets homomeric α7 neuronal nAChRs. Yet other α-conotoxins selectively block the muscle subtype of nAChR. Thus, it is increasingly apparent that α-conotoxins represent a significant resource for ligands with which to probe structure-function relationships of various nAChR subtypes.

The muscle subtype of nicotinic acetylcholine receptor (nAChR) is one of the best understood ligand-gated channels due in part to the availability of a large number of protein and small molecule ligands which serve as specific probes for this channel. The nAChR is a heteropentameric ion channel complex and is a member of a superfamily that includes glycine, GABA_A, and 5-HT_T receptors (1). The mammalian nAChR has the subunit composition (α1)β1δγ6 in developing muscle, and the γ subunit is replaced by an ε subunit in mature muscle. In mammalian neurons the situation is much more complex with at least seven α subunits, designated α2-α7 and α9 (in chick there is also an α8 subunit), and three β subunits, β2-β4. The α2, α3, and α4 subunits can each combine with β2 or β4 subunits to form functional channels when expressed in Xenopus oocytes, e.g. α2β2, α3β2, α2β4, etc. In addition, α7 and α9 subunits can be expressed as functional homolgomers in this system. Studies employing either nucleotide probes or antibodies indicate that each of these α and β subunits have a unique pattern of anatomical expression in the central nervous system (2). However, the precise structural composition and functional role of the different neuronal subtypes of nicotinic receptors are less well understood. The development of subtype-specific ligands will greatly aid progress in this area.

Although a number of valuable nicotinic antagonists have been described, few are highly subtype-selective, particularly in the case of neuronal nAChRs. α-Bungarotoxin, an alkaloid from the Chondrodendron tomentosum bush, used for centuries as an arrow poison to kill wild game, blocks both muscle and neuronal nAChRs (3). In addition it binds to all neuronal nicotinic receptors with more or less similar affinities (4). Likewise, dihydro-β-erythroidine, the hydrogenated derivative of erythroidine, isolated from trees and shrubs of the genus Erythrina is a competitive antagonist at both muscle and neuronal nAChRs (4). Lophotoxin, a small cyclic diterpene, is used by the soft shell coral Lophogorgia rigida to discourage its consumption by fish (5). This toxin forms a covalent bond with Tyr190 of the α-subunit of Torpedo nAChRs, irreversibly blocking the binding of Ach to the receptor (6, 7). Studies with nAChRs expressed in Xenopus oocytes reveal that this toxin blocks muscle nAChRs as well as α2β2, α3β2, and α4β2 neuronal nAChRs (8). Neosurugatoxin, a glycoside from the gastropod Babylonia japonica (9), potently but nonselectively blocks αβ2 nAChRs expressed in oocytes, where x is 2, 3, or 4 (8). The synthetically derived small molecules trimethaphan and mecamylamine discriminate between ganglionic and neuromuscular nAChRs and are used clinically as ganglionic blocking agents (3).

Numerous protein toxins which act at muscle nAChRs have been isolated from a variety of snake venoms and proven highly useful for studying nAChRs. Two toxins from the Taiwanese banded krait, Bungarus multiocinctus, have been particularly well characterized. The major nicotinic antagonist in this venom, α-bungarotoxin, in addition to blocking the muscle receptor, potently blocks α7 subunit-containing neuronal nAChRs (10). Methylylcystamine, an alkaloid toxin from the seeds of Delphinium brownii has markedly greater affinity for the 125I-α-bungarotoxin binding site in brain versus that in muscle demonstrating that these receptor subtypes can be pharmacologically distinguished (11). A minor component of Bungarus venom, α-bungarotoxin (also known as neuronal bungarotoxin, toxin F, or Bgt 3.1) preferentially blocks α3β2 receptors (8), although the presence of venom purification contaminants has led to inconsistent findings (8, 12). Unfortunately, due to limited availability of venom, this potent toxin is commercially unavailable at the present time.

A growing number of nicotinic antagonists have been isolated from the venom of the carnivorous marine snail Conus and are known as α-conotoxins. In contrast to snake α-toxins (60–80 amino acids), α-conotoxins are much smaller (12–25 amino acids), a feature which has allowed them to be readily chemically synthesized (13). α-Conotoxins, which target the

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† To whom correspondence should be addressed: University of Utah, 201 S. Biology, Salt Lake City, Utah 84112.

‡ The abbreviations used are: nAChR, nicotinic acetylcholine receptors; RPLC, reverse phase liquid chromatography; Fmoc, N-(9-fluorenyle)methoxycarbonyl.
muscle nAChRs are enjoying increasing use due to their recently discovered ability to differentiate between the two acetylcholine binding sites on the receptor. In the mouse muscle-derived BC3H-1 cell line α-conotoxins MI, GI, and SIA (respectively from Conus magus, Conus geographus, and Conus striatus) selectively bind to the ACh binding site at the α6 interface with more than 104-fold greater affinity than the site at the α7 interface (14, 15). With Torpedo nAChR, the situation is reversed. α-Conotoxins MI and GI bind at the α7 interface with approximately 2 orders of magnitude greater affinity than the α6 interface (15, 16). Like α-conotoxins MI, GI, and SIA, α-conotoxin E1, from Conus ermineus, prefers the α6 to the α7 interface of receptors in BC3H-1 cells, but with only 30-fold difference. In contrast to these other α-conotoxins, with Torpedo receptors, α-conotoxin E1 preferentially binds the α6 versus the α7 interface by a 400-fold difference in affinity and is the only ligand known to possess this selectivity (17). Thus, these α-conotoxins can serve as specific probes to investigate structure-function relationships of nAChRs (18).

There are approximately 500 species of Conus. Each of these predatory gastropods hunt prey from one of five different phyla, and all of these prey have cholinergic synapses (19). Thus, there is a potentially very wide diversity of nAChRs for conotoxins to target, and it is likely therefore that there are a comparatively wide spectrum of cholinergically active peptides in the venom of Conus. We are seeking to exploit this situation to develop a bank of peptides which act on specific subtypes of neuronal nicotinic receptors. By use of a bioassay involving intracellular injections into mice to guide purification, we previously isolated α-conotoxin IMI which, unlike other α-conotoxins, selectively targets α7, and to a lesser degree α9, nAChRs (20, 21). In the present study we used a much more specific screening assay to purify a novel nicotinic antagonist from C. magus venom. Voltage-clamped Xenopus oocytes expressing α3β2 nAChRs were used in the assay to isolate α-conotoxin MI. We report the structural characterization and nAChR subtype selectivity of this peptide.

MATERIALS AND METHODS

Peptide Isolation and Sequencing

Venom Extraction—Crude venom from dissected ducts of C. magus was collected in the Philippines, lyophilized, and stored at −70 °C until used. All reagents were precooled to, and extraction procedures were conducted at, 4 °C. Fifteen ml of 0.1% trifluoroacetic acid was added to 500 mg of lyophilized venom, and the mixture was vortexed for 20 min. This mixture was centrifuged at 17,000 × g for 20 min. The supernatant was transferred to a separate tube, and another 15 ml of 0.1% trifluoroacetic acid was added to the pellet which was then sonicated with a Sonifier (Branson instruments) at setting #4, vortexed for 10 min, and centrifuged as above. The supernatants were combined and filtered through a Whatman GF/C filter (Whatman, Ltd, Maidstone, UK), and this solution of this purified peptide (250 pmol) was diluted 3-fold with 0.1% trifluoroacetic acid, and the alkylated peptide further 25 min at room temperature in the dark. The solution was diluted 3-fold with 0.1% trifluoroacetic acid, and the alkylated peptide was loaded on the Brownlee column. After washing the column with 20% buffer B to allow the baseline to return to 10% of the initial reading, the peptide was eluted with the gradient described in Fig. 1, panel B.

Sequence Analysis—Sequencing was performed with Edman chemistry on an Applied Biosystems 477A Protein Sequencer at the Protein/DNA Core Facility at the University of Utah Cancer Center. Mass spectrometry was performed as described previously (17).

Peptide Synthesis

Linear Peptide—All amino acid derivatives were purchased from Bachem (Torrance, CA). The linear peptide chain was built on Rink amide resin from Bom Biochem (N-(9-fluorenylmethoxycarbonyl) procedures with 2-(1H-benzo[d]imidazole-1-yl)-1,1,3,3-tetramethyluronium tetrfluoroborate coupling, using an ABI model 430A peptide synthesizer. Side chain protection of non-Cys residues was with t-butyl (Bzl), Ser, and trityl (Trt, Hs). Orthogonal protection was used on cysteines Cys3 and Cys6 were protected as the stable Cys(S-acetamidomethyl), while Cys4 and Cys5 were protected as the acid-labile Cys(S-trityl). After assembly of the resin-bound peptide, the terminal Fmoc group was removed in situ by treatment with 20% piperidine in N-methylpyrrolidone. Linear peptide amide was cleaved from 93 mg of resin by treatment with 1 ml of anhydrous 4-vinylphenylacetic acid/30% piperidine in anhydrous acetic acid (1:100, v/v) for 2 h, diluted 100-fold with buffer B gradient over 50 min. Flow rate was 20 ml/min. This gradient was also used for all subsequent preparative RPLC purifications of the synthetic peptide.

Peptide Cyclization—To form a disulfide bridge between Cys2 and Cys6 (i.e. the first and third cysteines), the major peptide fraction from the preparative RPLC (see above) was diluted to 1 liter with H2O and solid Tris base was added to increase the pH to 7.6. The solution was placed in a 4-liter flask and gently swirled at room temperature for 38 h at which time the reaction was judged to be complete by analytical RPLC. The pH of the solution was decreased to a value of 2−3 (measured with pH paper) by the addition of 4 ml of trifluoroacetic acid. The monocyclic peptide was then purified by RPLC and collected in a volume of 45 ml. Removal of the S-acetamidomethyl groups and closure of the second disulfide bridge (Cys7-Cys16, i.e. the second and fourth cysteines) was carried out simultaneously by iodine oxidation. The 45 ml of RPLC eluent containing the monocylic peptide was dripped into a rapidly stirred 50-ml solution of 20 mM iodine in H2O/trifluoroacetic acid (1:1) which had been added to the reaction to a value between 7 and 8 as measured with pH paper. Seventy-five ml of 50 mM diithothreitol was added (final concentration 10 mM); the reaction vessel was flushed with argon, and the reaction incubated at 65 °C for 15 min. The solution was allowed to cool; 15 ml of 20% 4-vinyl pyridine in ethanol was added, and the solution was reacted for a further 25 min at room temperature in the dark. The solution was diluted 3-fold with 0.1% trifluoroacetic acid, and the alkylated peptide was loaded on the Brownlee column. After washing the column with 20% buffer B to allow the baseline to return to 10% of the initial reading, the peptide was eluted with the gradient described in Fig. 1, panel B.

Electrophysiology
cDNA Preparation—cDNA clones encoding nAChR subunits were provided by S. Heinemann and D. J. Ehson (Salk Institute, San Diego, CA). cDNA was transcribed using either RibMAX™ large scale RNA
production systems (Promega, Madison, WI) or an RNA transcription kit (Stratagene, La Jolla, CA). Diguanosine triphosphate (Sigma) was used for synthesis of capped cRNA transcripts according to the protocol of the manufacturer. Plasmid constructs of mouse and rat nAChR subunits were as described: α1, β1, γ, δ (22); α2 (23); α3 (24); α4 (25); α7 (26); α9 (26); β2 (27); and β4 (28).

cRNA Injection—cRNA was injected with a Drummond 10-μl micropipette (Drummond Scientific, Broomall, PA) essentially as described by Goldin (29). It was fitted with micropipettes pulled from glass capillaries provided for the microdispenser. The pipette tips were broken to an OD of 22–25 μm and back-filled with paraffin before mounting on the microdispenser. cDNA was drawn into the micropipette and 50 nl, containing 5 ng of cRNA of each subunit, was injected into each oocyte. In the case of muscle subunits, 0.5–2.5 ng of each subunit was injected.

Oocyte Harvesting—Oocytes were removed from Xenopus frogs, cut into clumps of 20–50 oocytes, and placed in a 50-ml polypropylene tube (Sarstedt) containing 580 units/ml type 1 collagenase (Worthington) in OR-2 (82.5 mM NaCl, 2.0 mM KCl, 1.0 mM MgCl₂, and 5 mM HEPES, pH 7.3). The tube was incubated for 1–2 h on a rotary shaker rotating at 50 rpm. Half-way through the incubation, the solution was exchanged with fresh collagenase solution. The oocytes were then washed with six to eight 50-ml volumes of OR-2, transferred to a 60 × 15-mm Petri dish containing ND-96 (96.0 mM NaCl, 2.0 mM KCl, 1.8 mM CaCl₂, 1.0 mM MgCl₂, 5 mM Hepes, pH 7.1–7.5)/Pen/Strep/Gent (100 units/ml penicillin G (Sigma), 100 μg/ml streptomycin (Sigma), and 100 μg/ml gentamycin (Life Technologies, Inc., Grand Island, NY)). The oocytes were visually examined and only healthy appearing oocytes were transferred to a second dish containing ND-96 and antibiotics. Oocytes were injected 1–2 days after harvesting and recordings were made 1–7 days after injection.

Voltage-clamp Recording—An injected oocyte was placed in a 30-μl recording chamber consisting of a cylindrical well (4 mm diameter × 2 mm deep) fabricated from Syglgard, and gravity-perfused with either ND96 or ND96 containing 1 μM atropine (ND96A) at a rate of 1 ml/min. All toxin solutions also contained 0.1 mg/ml bovine serum albumin to reduce nonspecific adsorption of toxin. The perfusion medium could be switched to one containing toxin or acetylcholine (ACh) by use of a distributor valve (SmartValve, Cavro Scientific Instruments, Sunnyvale, CA) and a series of three-way solenoid valves (model 161TO31, Neptune Research, Northboro, MA). ACh-gated currents were obtained with a two-electrode voltage-clamp amplifier (model OC-725B, Warner Instrument Corp., Hamden, CT) set for "fast" clamp and with clamp gain at maximum (× 2000). Glass microelectrodes, pulled from fiber-filled borosilicate capillaries (1 mm outer diameter × 0.75 mm inner diameter, WPI Inc., Sarasota, FL) and filled with 3 M KCl, served as voltage and current electrodes. Resistances were 0.5–5 megohm for voltage, and 0.5–2 megohm for current electrodes. The membrane potential was clamped at −70 mV, and the current signal, recorded through virtual ground, was low-pass filtered (5 Hz cut-off) and digitized at a sampling frequency of 20 Hz. The solenoid perfusion

**Fig. 1.** Purification of α-conotoxin MII by RPLC. Panel A, filtrate of venom extract (38.2 ml) was loaded onto a semi-preparative Vydac C18 column with 0% buffer B and subsequently eluted using a gradient system. The gradient was 0–15% buffer B/15 min, then 15–39% buffer B/72 min, then 39–65% buffer B/15 min, then 65–100% buffer B/5 min and held at 100% buffer B for 2 min. Flow rate was 5 ml/min. Panel B, fractions indicated by the arrow in Panel A were diluted with 2 volumes of 0.1% trifluoroacetic acid and re-chromatographed as described in Panel B to obtain the final purified product. A 5-ml sample loading loop was used in all chromatography. Buffer A, 0.1% trifluoroacetic acid; and buffer B, 0.1% trifluoroacetic acid, 60% acetonitrile. Absorbance was monitored at 220 nm.

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2 J. Boulter, unpublished data.
The concentration of ACh was 1 mM for oocytes expressing α7, 1 mM for α1, and 300 μM for all others. The ACh was diluted in ND96A for all except α7, in which case the diluent was ND96. For control responses, the ACh pulse was preceded by perfusion with ND96 (for α7) or ND96A (all others). No atropine was used with oocytes expressing α7, since it has been demonstrated to be an antagonist of these receptors (30). For responses to toxin (test responses), the oocyte was perfused with toxin solution until equilibrated (generally 5 min, but up to 25 min at lower toxin concentrations) before the ACh pulse was applied. All ACh pulses contained no toxin, for it was assumed that little, if any, bound toxin would have washed away in the brief time (<2 s) it takes for the responses to peak (see Fig. 3). The peak amplitudes of the ACh-gated current responses were measured by the virtual instrument. The average of three control responses just preceding a test response was used to normalize the test response to obtain % response. Each data point of a dose-response curve represents the average ± S.E. of at least three oocytes.

Fig. 2. Comparison of natural and synthetic α-conotoxin MII by RPLC. Native and synthetic peptide have similar elution times when chromatographed separately and co-migrate when coinjected on RPLC. RPLC conditions were as described in the legend to Fig. 1, panel B, except that a 1-ml sample loading loop was used. Absorbance was monitored at 220 nm. Maximum OD readings were 0.0031, 0.16, and 0.012 absorbance units in the first, second, and third panels, respectively.

Fig. 3. α-Conotoxin MII blocks ACh responses in oocytes expressing α3β2 nicotinic acetylcholine receptors. Oocytes expressing α3β2 nAChRs were voltage-clamped and the response to a 1-s pulse of ACh was measured (see “Materials and Methods”). Panel A, 0.5 nM α-conotoxin-MII blocks 45% of the ACh-induced response. Panel B, in a different oocyte, 20 nM toxin blocks 98% of the ACh-induced response.
tion, and the monocyclic peptide was purified by RPLC. The acid-stable acetamidomethyl group was next removed from Cys5 and Cys16 (i.e. the second and fourth cysteines), and the disulfide bridge closed by rapid iodine oxidation and the bicyclic peptide purified by RPLC. Peptide yield was 41.7 nmol/mg peptide resin.

The order of RPLC elution of the synthetic peptides is of note: linear first, followed by monocyclic and bicyclic last. With the formation of each disulfide bridge, α-conotoxin MII becomes increasingly hydrophobic. This is exactly the opposite behavior from α-conotoxin E1, where the formation of each disulfide bond results in decreased retention time on RPLC (17), and may indicate that the disulfide bridges in α-conotoxin MII force hydrophobic residues to face outward. Synthetic peptide comigrated with native on RPLC (Fig. 2). Liquid secondary ionization mass spectrometry of synthetic α-conotoxin MII was consistent with the amidated sequence (monoisotopic MH+; calculated 1710.65, observed 1710.8). Lyophilization or bath application of small (fmol—pmol) amounts of α-conotoxin MII resulted in apparent loss of peptide (data not shown). The hydrophobic nature of the peptide may account for this problem which was minimized by the use of carrier protein (bovine serum albumin) in all solutions and continuous perfusion rather than bath application of peptide.

Electrophysiology—α-conotoxin MII purification by RPLC was guided by an assay which used Xenopus oocytes expressing α3β2 receptors. RPLC fractions were tested for their ability to block the ACh-induced response in this assay. We also examined the effect of the toxin on other nACHR subunit combinations expressed in oocytes. Both native and synthetic toxin blocked the α3β2 nACHRs with equal potency (data not shown). Due to very limited availability of native toxin, synthetic toxin was used for all subsequent experiments. Synthetic α-conotoxin MII showed dose-dependent block of α3β2 receptors at low nanomolar concentrations (Fig. 3). This block slowly reversed with washing (Fig. 4). α-Conotoxin MII blocks α3β2 nACHRs with an IC50 of 0.5 nm, with an apparent Hill coefficient, nH, of 0.8 (Fig. 5). α-Conotoxin MII was also tested on other nACHR subunit combinations. Results are shown in Fig. 5 and indicate that α-conotoxin MII is 2–4 orders of magnitude more potent at α3β2 nACHRs than at other nACHR subtypes.

We have previously shown that α-conotoxin MII (also from C. magus venom), α-conotoxin GI, and α-conotoxin lM1 have no effect on α3β2 receptors at up to 5 μM concentration (21). Thus, α-conotoxin MII is the only conotoxin known to potently block this neuronal receptor subtype.

In Vivo Activity—Intraperitoneal injections of 10 nmol of α-conotoxin MII into 8-10-g mice did not result in any signs of paralysis (n = 3). This is in contrast to α-conotoxin MII, 0.67 nmol of which kills a 20-g mouse in 20 min (32). Intramuscular injection of 5 nmol of α-conotoxin MII into fish did not result in any signs of paralysis (n = 3). This is in contrast to α-conotoxin M1 where 0.5 nmol is paralytic.

DISCUSSION

nACHR Selectivity—Xenopus oocytes expressing mammalian neuronal α3β2 nACHRs were used in an assay which successfully guided the isolation of the novel 16-residue peptide, α-conotoxin MII. This is significant in that it is the first α-conotoxin known to target α3β2 receptors. Most previously reported α-conotoxins target the muscle nACHR. Exceptions are α-conotoxin lM1, which selectively blocks homomeric α7 and α9 receptors (20, 21), and α-conotoxins PnLA/B, which block molluscan neuronal nACHRs (33). We have shown elsewhere that α-conotoxins M1 and GI potently target muscle nACHRs expressed in Xenopus oocytes, but are inactive at all neuronal nACHRs tested, including α3β2 receptors (21). As demonstrated in this report, α3β2 receptors are blocked by α-conotoxin MII with an IC50 of 0.5 nm. The effectiveness of the toxin on the other nACHR subunit combinations tested is 2–4 orders of magnitude less. Thus, α-conotoxin MII has an entirely unique activity profile among the α-conotoxins (Table I), and represents a potent and selective new probe for studying nACHRs. Notably, the small size of α-conotoxin MII has allowed it to be chemically synthesized and thus readily available.

Structural Relationships among α-Conotoxins—Reported
α-conotoxins can be classified into two main groups based on the spacing of the cysteine residues. One group has a “3,5 spacing” where the numerals indicate the number of amino acids between the second and third Cys and the third and fourth Cys, respectively (see Table II). The other group, which includes α-conotoxin M11, has a “4,7 spacing.” Individual toxins from both groups can potently block the muscle nAChR, suggesting that it is not the Cys spacing which is responsible for α-conotoxin M11’s selectivity. Aside from the Cys residues, the only completely conserved residue in all reported α-conotoxins is a proline between the second and third Cys. The other non-Cys residues in α-conotoxin M11 are strongly divergent from all other α-conotoxins. Of the non-Cys residues in the 4,7 group, α-conotoxin M11 from *C. magus* shares only 4 of 12 residues with α-conotoxin PnB from *C. pennaceus* and only 1 of 12 residues with α-conotoxin EI from *C. ermineus*. Furthermore, except for the proline, α-conotoxin M11 shares little if any homology with α-conotoxin M1 (which has a 3,5 spacing) although both are from the same Conus species. However, despite the difference in Cys spacing and strong divergence in other amino acids, the disulfide bridges are exactly analogous in spite the difference in Cys spacing and strong divergence in other amino acids, the disulfide bridges are exactly analogous. This suggests that the disulfide bridges are determined first and that the Cys residues are added second. Some α-conotoxins have a distinctly different structure including three disulfide bridges instead of two (34).

Multiple nAChR-targeted toxins have previously been isolated from two other Conus species, *C. geographus* and *C. striatus* (see Table II). In both of these cases, however, the α-conotoxins have considerable structural homology and all target the muscle nAChR in contrast to the M1 and M11 peptides isolated from *C. magus* that differ substantially in both structure and function. It will be of interest to determine which residues in M11 confer αβ2α2 nAChR selectivity and which in M11 confer αβ2β2 selectivity.

**Biological Role—**Injection of venom by Conus snails results in prey immobilization and capture. Nevertheless, the majority of the 100–200 peptides present in the venom of fish-hunting Conus do not induce paralysis when injected into fish. The functional targets and roles of these non-paralytic peptides are under investigation. However, all previously reported α-conotoxins from fish-hunting Conus do cause rapid paralysis when injected into fish. α-Conotoxin M11 is unique in not causing paralysis in this assay. It has recently been shown that only as few as three amino acid substitutions in the γ or δ subunit of mouse nAChR can result in a 10-fold change in the affinity of this receptor for α-conotoxin M1 (18). It has also been shown that a single amino acid substitution in α-conotoxin S1 increases its affinity for mouse muscle nAChRs by 2 orders of magnitude (35). It is possible, therefore, that α-conotoxin M11 does potently target the muscle nAChR of its natural tropical fish prey, and that a few amino acid substitutions in the goldfish nAChR used in our assay may be responsible for the observed substantial differences in toxin potency. Poor dispersal of the more hydrophobic α-conotoxin M11 might also lead to an apparent lack of activity in our assay. Since *C. magus* already has a toxin which potently blocks muscle nAChRs in the form of α-conotoxin M11, another possibility is that *C. magus* uses α-conotoxin M11 to selectively target ganglionic or adrenergic nAChRs in fish to lessen the sympathetically mediated fight or flight response. In frog, α-conotoxin M11 blocks ganglionic neurotransmission. A related example may be neosurgatoxin. This glycoside, isolated from mid-gut gland of the Japanese ivory shell, appears to be responsible for human poisonings following ingestion of this carnivorous gastropod (9). Poisoning symptoms are consistent with blockade of autonomic ganglia. Like α-conotoxin M11, neosurgatoxin preferentially targets neuronal versus muscle nAChRs. In contrast, however, neosurgatoxin is non-selective among αβ2α2 nAChRs (4, 8).

The discovery of α-conotoxin M11, which differs substantially in both structure and function from other α-conotoxins, provides further evidence of the enormous diversity of nAChR-targeted toxins present in *Conus*. This report demonstrates the feasibility of using specific nAChR subunit combinations expressed in oocytes as a functional screen to initially detect and ultimately guide the purification of these peptides.

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**TABLE II**

| α-Ctx | Sequence | Species | Prey | ref |
|-------|----------|---------|------|-----|
| M11   | GCCSPEPRAVISLHNC* | magus   | fish | This report |
| E1    | RDDORCIDCPCKRCFIC* | ermineus | fish | (17) |
| PnB   | GCCSLIPQAOHHRDC* | pennaceus | mole sole | (32) |
| PnA   | GCCSLIPQAOHHRDC* | pennaceus | mole sole | (33) |
| G1    | ECRFEAQF00042C* | geographic | fish | (26) |
| G1A   | ECRFEAQF00042C* | geographic | fish | (26) |
| G1I   | ECRFEAQF00042C* | geographic | fish | (26) |
| M1   | GECEQEAQFC00042C* | magus | fish | (32, 37) |
| S1    | ICRFEAQF00042C* | cirtainus | fish | (28) |
| S1A   | YCEPFEAQF00042C* | cirtainus | fish | (29) |
| S1I   | GCOSPEPRAVISLHNC** | cirtainus | fish | (40) |

**A single or double asterisk indicates that the COOH-terminal carboxy-tetraacyclic glutarate group is known to be either amidated or the free acid, respectively.**
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\(\alpha\text{-Conotoxin MII Targets } \alpha3\beta2 \text{nAChRs}\)
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