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Conus peptides: novel probes for nicotinic acetylcholine receptor structure and function

J. Michael McIntosh [a,b,*], Stephanie Gardner [a], Siqin Luo [a], James E. Garrett [c], Doju Yoshikami [a]

[a] *Department of Psychiatry, University of Utah, Salt Lake City, UT 84112 USA*
[b] *Department of Biology, University of Utah, Salt Lake City, UT 84112, USA*
[c] *Cognetix, Salt Lake City, UT, 84108, USA*

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Abstract

Conus is a genus of predatory marine snails that uses venom to capture prey. Among the neurotoxins widely utilized by the cone snails are the α-conotoxins which are disulfide-rich peptides that target muscle or neuronal subtypes of nicotinic acetylcholine receptors. The small size and receptor subtype specificity of these peptides make them particularly useful for characterizing both native and heterologously expressed nicotinic receptors. In this report, we demonstrate that α-conotoxin MII potently blocks β3-containing neuronal nicotinic receptors. Furthermore, initial evidence suggests that subpopulations of α3β2β3-containing receptors are differentially sensitive to α-conotoxin MII. Thus, α-conotoxin MII promises to be a useful tool for studying neuronal nicotinic receptors containing the β3 subunit.

Keywords: α-Conotoxin MII; *Conus*; Nicotinic acetylcholine receptor, human; β3 subunit

1. Introduction

Plants and animals historically have been a rich source of antagonists of nicotinic acetylcholine receptors. One of the more recently explored sources is the marine snail *Conus*, a venomous predator. There are over 500 species of cone snails; each venom thus far examined contains multiple compounds targeted to neuronal nicotinic receptors. Many of these compounds are disulfide-rich peptides. Their small size has made them amenable to chemical synthesis (see McIntosh et al., 1999 for review).

The initial α-conotoxins characterized (e.g., α-conotoxin GI and α-conotoxin MI) potently block muscle but not neuronal nicotinic receptors (Luetje et al., 1990; Johnson et al., 1995). With regard to muscle nicotinic receptors, in mammalian muscle, these α-conotoxins show five orders-of-magnitude selectivity for the α/δ vs. the α/γ subunit interface (Sine et al., 1995). More recently, α-conotoxins that target neuronal nicotinic receptors have been characterized. α-Conotoxin ImI was isolated from a worm-hunting cone; ImI selectively blocks the mammalian α7 receptor (Johnson et al., 1995). The α7 subtype of receptor is notable for its rapid desensitization. It is of interest that α-conotoxin ImI also blocks apparently rapidly desensitizing receptors populations in invertebrates. In *Aplysia*, α-conotoxin ImI selectively blocks the rapidly desensitizing portion of the acetylcholine-gated chloride current (Kehoe and McIntosh, 1998). In locusts, α-conotoxin ImI blocks the rapidly desensitizing portion of the acetylcholine-induced current in thoracic ganglion (Van den Beukel et al., 1998). α-Conotoxin MII, isolated from a fish-hunting cone, selectively blocks the rat α3β2 receptor with subnanomolar affinity (Cartier et al., 1996). In contrast to α-conotoxin ImI, which blocks rapidly desensitizing currents, α-conotoxin MII selectively blocks the slowly decaying current in chick ciliary ganglion (Ullian et al., 1997). In frog sympathetic ganglia, α-conotoxin MII and α-conotoxin ImI differentially block synapses on B vs. C

* Corresponding author. Department of Biology, University of Utah, Salt Lake City, Utah 84112, USA. Tel.: +1-801-585-3622; fax: +1-801-581-4668.

E-mail address: mcintosh@bioscience.utah.edu (J.M. McIntosh).

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neurons (Tavazoie et al., 1997). In addition to fish- and worm-hunting cone snails, α-conotoxins have also been isolated from mollusc-hunting snails. One example is α-conotoxin AuIB which selectively targets rat α3β4 neuronal receptors (Luo et al., 1998) and putative α3β4-containing neuronal nicotinic receptors present in rat habenula neurons (Quick et al., 1999). α-Conotoxins MII and AuIB have been used to selectively block nicotine-induced dopamine release and nicotine-evoked norepinephrine release, respectively (Kulak et al., 1997; Kaiser et al., 1998; Luo et al., 1998).

In this study we report that, in addition to α3β2 receptors, α-conotoxin MII potently blocks α3β2β3 receptors expressed in *Xenopus* oocytes. Initial results also suggest that there are distinct populations of α3β2β3 receptors that are differentially sensitive to α-conotoxin MII.

2. Materials and methods

2.1. Materials

α-Conotoxins were synthesized as previously described (Cartier et al., 1996). Toxins were stored in lyophilized form at −20° C until use.

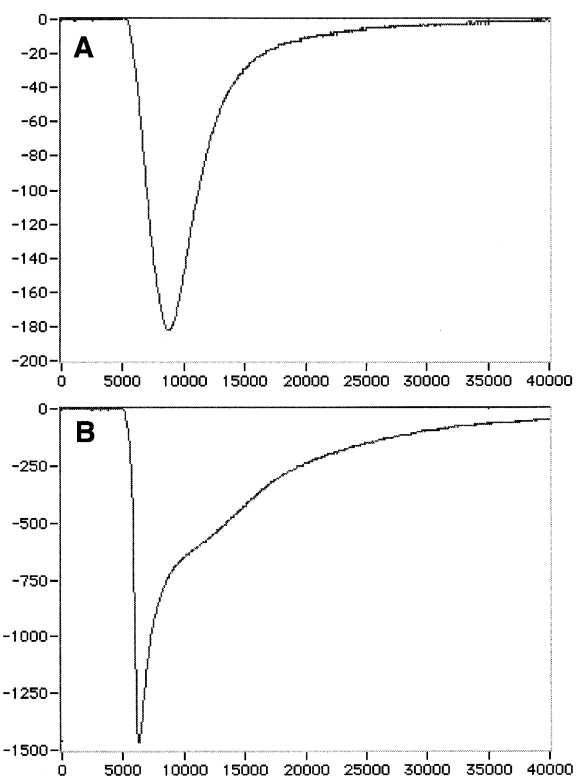

Fig. 1. ACh-induced current from nAChRs expressed in *Xenopus* oocytes. Vertical axis, I_{ACh} in nA; horizontal axis, time in ms. A 1-s pulse of 300 μM ACh was applied at $t = 5$ s. Response of oocyte expressing human α3β2 subunits (panel A) and α3β2β3 subunits (panel B).

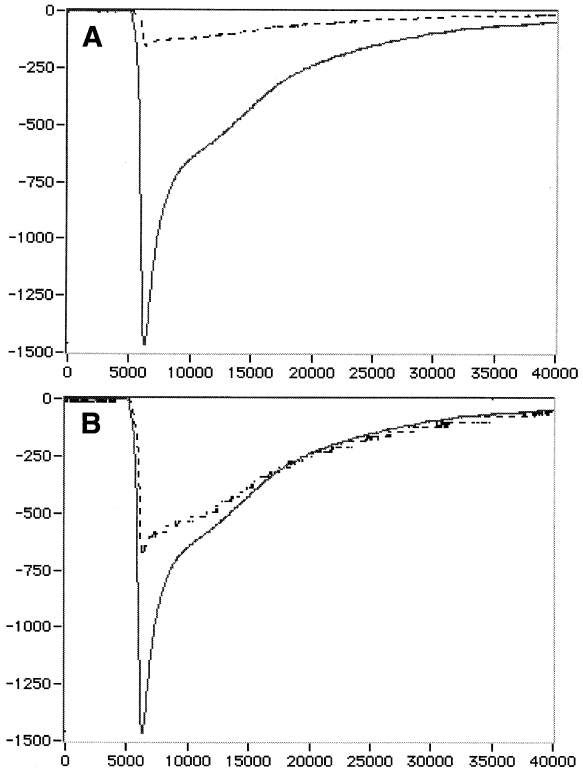

Fig. 2. Effect of α-conotoxin MII on α3β2β3 receptors. Graph axes and ACh-application are as in Fig. 1. Panel A, I_{ACh} before (solid trace) and during (dashed trace) exposure to 10 nM α-conotoxin MII. Panel B, same as panel A except the trace obtained in the presence of α-conotoxin MII has its vertical axis expanded 4.5-fold (such that the late falling phases of both responses are superimposed). Note that the early, rapidly decaying portion of the current is preferentially eliminated by MII.

2.2. Cloning of neuronal nicotinic receptor subunits

Clones of the human nicotinic acetylcholine receptor subunits were generated by reverse transcription–polymerase chain reaction (PCR). Sequences in the 5′ and 3′ untranslated regions of each of the neuronal nicotinic receptor mRNAs were used to design PCR primers to amplify the complete open reading frame of each subunit. Restriction enzyme sites were incorporated into the PCR primers to facilitate directional cloning into the pBluescript II SK$^-$ vector. PCR amplification was performed with a proofreading polymerase mixture (Bio-X-Act polymerase; Bioline, London, UK). PCR products were cloned into the plasmid vector and confirmed by DNA sequencing. To obtain cRNA of neuronal nicotinic receptor subunits for injection into *Xenopus* oocytes (see below), 5′ capped sense-strand RNA was transcribed in vitro using T7 RNA polymerase according to published procedures (Melton et al., 1984). Integrity of the cRNAs was assessed by denaturing gel electrophoresis.

2.3. Electrophysiology

Oocyte injection and recording were performed as previously described (Luo et al., 1998). α3β2β3 oocytes

Table 1

α-Conotoxins	Sequence	Source	Target
MI	GR**C**C**H**PA**C**GKNYS**C**[a]	Fish hunter	α1β1δγ
ImI	G**CC**SDPR**C**AWR**C**[a]	Worm hunter	α7
MII	G**CC**SNPV**C**HLEHSNL**C**[a]	Fish hunter	α3β2; α6-containing receptor (chick); β3-containing receptor
AuIB	G**CC**SYPP**C**FATNPD**C**[a]	Mollusc hunter	α3β4

[a]Amidated C-terminus.

were each injected with 0.5, 0.5 and 1.0 ng of each receptor subunit cRNA respectively.

3. Results

Oocytes injected with cRNA for the β3 subunit alone, the α3 subunit alone, the β2 subunit alone, the β2 and β3 subunits in combination, and the α3 and β3 subunits in combination, all failed to express active receptor as tested with application of 300 μM acetylcholine. In contrast, oocytes injected with either α3β2 or α3, β2 and β3 cRNA reliably expressed active receptor. In oocytes expressing α3β2 receptors, acetylcholine elicited a relatively symmetrical-shaped response (Fig. 1A). In oocytes injected with α3 + β2 + β3 cRNAs, acetylcholine consistently produced what appeared to be a multicomponent response (Fig. 1B).

α-Conotoxin MII (10 nM) blocks approximately 93% the acetylcholine-induced response in α3β2-expressing oocytes (data not shown). α-Conotoxin MII also potently blocks receptors expressing α3β2β3 receptors (see Fig. 2A). Examination of the acetylcholine-induced response in the presence of 10 nM α-conotoxin MII suggests that MII preferentially blocks the initial rapidly decaying component of the response (see Fig. 2B).

4. Discussion

α-Conotoxins from different species of cone snails have been used to selectively antagonize neuronal nicotinic receptors with different subunits (see Table 1). It should be noted that the effectiveness of these toxins for a given subtype of nicotinic receptor may vary according to the species of the organism producing the receptor.

In this report, we have shown that the human β3 subunit combines with α3 and β2 subunits to form functional receptors. In addition, we have shown that α-conotoxin MII potently antagonizes receptor function. Examination of the shape of the acetylcholine-induced current suggests that oocytes injected with α3 + β2 + β3 subunit cRNA express a mixed population of receptors; the rapidly desensitizing subpopulation appears to be particularly sensitive to α-conotoxin MII.

Noteworthy is the recent report that immunoprecipitated nicotinic receptors containing the α6 subunit from chick are sensitive to α-conotoxin MII. The amino acid sequence of the chick α6 subunit shares approximately two-thirds identity to that of the α3 subunit. It is notable that approximately one-half of the α6-containing receptors immunoprecipitated also contained a β3 subunit (Vailati et al., 1999).

Acknowledgements

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