

Analogues of α -conotoxin PnIC are selective for $\alpha 7\beta 2$ over $\alpha 7$ -only subtype nicotinic acetylcholine receptors

Paul Whiteaker,¹ Andrew A. George,¹ Sabin J. John,² Linda M. Lucero,³ Jason B. Eaton,³ Ekta Jaiswal,³ Yiwei Cao,⁴ Wonpil Im,⁴ and Joseph M. McIntosh⁵

¹Virginia Commonwealth University; ²University of Bath; ³Barrow Neurological Institute; ⁴Lehigh University; and ⁵University of Utah

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Objective/Aim/Hypothesis: The objective of this work is to discover and characterize the first ligands able to identify selectively nicotinic acetylcholine receptors assembled from $\alpha 7$ and $\beta 2$ subunits ($\alpha 7\beta 2$ -nAChR). Basal forebrain cholinergic neurons (BFCNs) express $\alpha 7\beta 2$ -nAChR. Elevated concentrations of oligomeric amyloid- β (associated with early Alzheimer's disease) appear to mediate BFCN neuronal dysfunction. Expression of $\alpha 7\beta 2$ -nAChR by additional important cholinergic and GABAergic neuronal circuits of the central nervous system has been observed. However, further studies are stymied by a lack of ligands that can positively identify $\alpha 7\beta 2$ -nAChR from the related and more widespread $\alpha 7$ -only homomeric nAChR subtype. This problem arises since $\alpha 7$ -only- and $\alpha 7\beta 2$ -nAChR share identical agonist binding sites, located at $\alpha 7/\alpha 7$ subunit interfaces, that are the targets of most current ligands. We hypothesized that new α -conotoxin (α -Ctx) ligands may instead selectively inhibit $\alpha 7\beta 2$ -nAChR via $\alpha 7\beta 2$ subunit interfaces.

Design/Approach/Methods: Two-electrode voltage clamp (TEVC) electrophysiology was used to screen $\gg 500$ novel α -Ctxs for selectivity towards $\alpha 7\beta 2$ - over $\alpha 7$ -only-nAChR functionally expressed in *Xenopus* oocytes. Kinetics analysis was used to probe the subtype selectivity and mechanism of α -Ctx antagonism. Molecular dynamics (MD) simulations were used to identify amino-acid residues at putative $\alpha 7\beta 2$ subunit α -Ctx binding sites. Site-directed mutagenesis was used to probe these hypothesized sites. TEVC electrophysiology was also used to determine α -Ctx activity at non- $\alpha 7$ nAChR subtypes.

Results: We discovered α -CtxPnIC analogs that selectively antagonize $\alpha 7\beta 2$ - over $\alpha 7$ -only-nAChR. Kinetics analysis showed that association rates were similar across α -CtxPnIC analogs, and between $\alpha 7\beta 2$ - and $\alpha 7$ -only-nAChR subtypes. Slower disassociation from $\alpha 7\beta 2$ - vs. $\alpha 7$ -only-nAChR mainly drove selectivity towards $\alpha 7\beta 2$ -nAChR. The α -CtxPnIC [S4R] and [L10Y] analogs were the most selective towards $\alpha 7\beta 2$ -nAChR (18- and 57-fold vs. $\alpha 7$ -only-nAChR, respectively). MD identified two sets of $\beta 2$ subunit residues at the putative $\alpha 7\beta 2$ subunit interface for α -CtxPnIC analogs that differed from those at the known $\alpha 7/\alpha 7$ subunit interface competitive agonist site. Mutating either set of $\beta 2$ subunit residues to their $\alpha 7$ subunit equivalents partially reduced α -CtxPnIC [S4R] selectivity towards $\alpha 7\beta 2$ -nAChR. Activity of α -CtxPnIC analogs was generally low at non- $\alpha 7$ -nAChR subtypes; data across analogs suggested approaches to decrease off-target affinity further.

Conclusions: We have identified the first ligands with selectivity towards $\alpha 7\beta 2$ -nAChR and proved a prototypical example of non-competitive antagonism by α -Ctxs. This discovery profoundly expands the scope of application of α -Ctx ligands (which have already provided important nAChR research and translational breakthroughs). Further development of α -CtxPnIC analogs will enhance $\alpha 7\beta 2$ -nAChR selectivity, providing opportunities for basic and translational scientific breakthroughs related to nAChR biology, Alzheimer's disease, and cholinergic contributions to cognition.

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