GLADTIER: A high throughput analysis for characterizing toxin receptor interactions for disruption or reversibility of toxin activity

Vishaka Santosh[1,2], Gabrielle Rizzo[1], Conor Jenkins[1], Andrew Walz[1], Brooke Ahern[1]
[1]U.S. Army Combat Capabilities Development Command Chemical Biological Center, Aberdeen Proving Ground, MD, [2]Excet Inc., 6225 Brandon Ave, Suite 360, Springfield, VA 22150

Introduction

Conotoxins are short, 10-30 amino acid residue long neurotoxic peptides that can be isolated from the venom of cone snails; these peptides affect numerous neurotransmitter receptors and ion channels, particularly those within the neuromuscular junction. In response to an action potential, voltage-gated calcium channels open to allow the flow of calcium into the nerve terminal; this promotes presynaptic vesicles to release their contents of acetylcholine (ACh) into the synaptic cleft to trigger action potential transmission to the muscle via nicotinic acetylcholine receptors (nAChR). Inhibition of nAChR, such as by α-conotoxins results in muscle paralysis, and therefore potential death.

Here, we propose a Guided Linking Application to Decide Threat Interaction Effect Radius (GLADTIER), a method that combines recent advancements in patch-clamping and cross-linking mass spectrometry to fully characterize structural and functional changes in response to conotoxin exposure. This will provide a high throughput investigation and analysis method that can be applied to a library of other toxins and their specific ion channels targets. To establish and validate this method of analysis, we first investigated nAChR because of the recent structural/functional information published; this information will then be applied to synthesize potential peptides that could disrupt channel inhibition by its respective conotoxin.

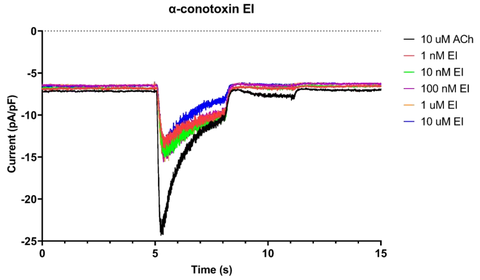
Methods

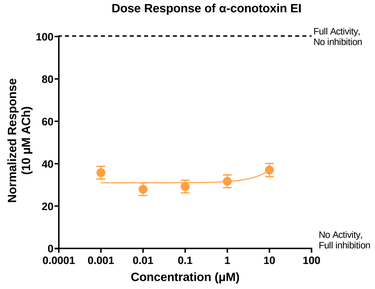
HEK cells expressing the muscle subtype of nAChR ($\alpha_1\beta_1\delta\varepsilon$) were functionally validated through automated patch-clamping (Sophion qPatch). Current was induced with acetylcholine, and then cells were exposed to multiple concentrations of α-conotoxin EI to measure a dose response curve.

Unexposed cells were also analyzed through crosslinking mass spectrometry to validate the structure of the nAChR was correct, and account for background that could interfere with conotoxin/receptor interaction network interpretation.

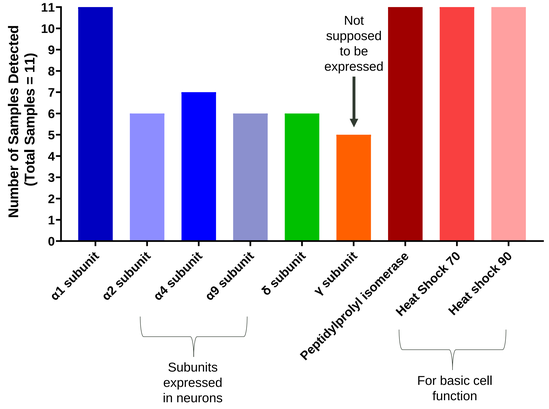
DEVCOM CBC @ DTRA CBD S&T Conference
Scan the QR Code to view all of CBC's
2022 DTRA CBD S&T Conference materials
https://cbc.devcom.army.mil/cbdst-conference/

Results





α-conotoxin EI did not completely inhibit acetylcholine induced current. Representative current traces (left) from one cell demonstrate that introduction of conotoxin partially inhibited channel activity (colored lines for concentrations of conotoxin) compared to baseline (black line, just acetylcholine). The dose response curve (right) measured for α-conotoxin EI demonstrates a relatively consistent percentage of inhibition across all concentrations tested, ~35% channel activity remaining.



nAChR-expressing cells express multiple α subunits; do not express all critical subunits. Eleven different samples containing technical replicates of cells are shown, with columns representing how many of those samples displayed the labeled protein. Multiple α subunits from neurons are expressed despite being described as a cell line that only expressed the muscle subtype, α_1. δ is only found in some of the samples, and γ is also found despite the description of the cell line. β1 and ε were not detected.

Conclusions

α-conotoxin EI targets the muscle subtype of the nAChR; however our results currently demonstrate that exposure to α-conotoxin EI does not induce complete inhibition. These results raise two main concerns:

1) Our nAChR cell line potentially expresses more than just the muscle subtype of the nAChR.
- α-conotoxin EI mainly inhibits the muscle subtype, the heterogeneity in expression might explain why we do not see complete inhibition of acetylcholine-induced current.
- The expression of neuronal and muscle subtype nAChR could give competing interaction networks, thus confound which binding sites are critical.

2) α-conotoxin EI binds specifically to the δ and γ subunits of the nAChR.
- Cells are made to express the δ subunit; not all samples expressed this subunit and could explain why we only have partial inhibition.
- The detection of the γ subunits in some of the samples could also explain that we see partial inhibition
- If the ε subunit was expressed instead, it is possible we would not see any inhibition.

Future Directions

- Purification of nAChR from cells to validate what is expressed in the cells, consult with vendor on what we see and what they see in their validation
- Ordered α-conotoxin EI from different vendor for further validation against our cells

Acknowledgements: Funding was provided by the U.S. Army via the In-house Laboratory Independent Research Program (PE 0601101A Project 91A) at the Combat Capabilities Development Command (DEVCOM) Chemical Biological Center. The views expressed in this abstract are those of the authors and do not necessarily reflect the official policy or position of the Department of Defense or the U.S. Government.