TOXIN MEDICAL COUNTERMEASURES - DEVELOPMENT OF NOVEL, BROAD-SPECTRUM COUNTERMEASURES FOR TOXIN EXPOSURE

Optimization of Production and Purification of Nicotinic Acetylcholine Receptors (nAChR) for Toxin-binding Analysis

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Introduction

The nicotinic acetylcholine receptor (nAChR) is the target of a variety of neurotoxins from an extensive range of algae, plants and animals. nAChR binding neurotoxins include α -conotoxins, a Health and Human Services select agent, and strychnine, a readily-available pest control poison. The nAChR neurotoxins have a diversity of structures but all bind to just seventeen transmembrane nAChR subunit sequences found in mammals. Neuronal nAChRs exist as homo-pentamers of α7, α8, and α9, or as hetero-pentamers of α2–α6 in combination with β2–β4, or α9- α10 combinations. Structural studies on transmembrane proteins are difficult, and while characterizing only the extracellular domains of nAChRs has shed light on some aspects of toxin binding, there is no full structure available for a toxin-bound nAChR. Additionally, the ligand binding pocket (LBP) for the agonists of the receptor exists at the interface of the two adjacent subunits, such that the correct composition of heterogeneous receptor subunits is vital for understanding nAChR:toxin pharmacology, but expression of heteromers in the correct composition has been challenging.

Due to the potential impact of nAChR toxins, it is important to develop countermeasures that target the toxins. However, many nAChR toxins are small and weakly immunogenic, and thus are often not effectively targeted by traditional antibody therapies, leading to a desire to develop novel broad-spectrum anti-neurotoxin therapies. The development of therapeutics has been impaired by a lack of detailed characterization of toxin-nAChR interactions.

Methods

To overcome this challenge, we have developed protocols for nAChR expression in common protein-production human and insect cell lines. The human endothelial kidney 293 Freestyle cell line was transfected to transiently express various mammalian nAChRs, and optimization of the process included additional transfection of neuronal chaperones to produce neuronal nAChRs. Baculovirus infection of insect cell lines was used for high-yield expression of acetyl choline binding proteins (AChBPs), derived from molluscs. Insect cell lines impart glycosylation on the produced proteins, unlike bacterial protein production systems, making them a valuable system for soluble protein production. The produced AChBPs serve as an alternative system to producing full length mammalian nAChRs.

Results

We have found altering identity and location of the affinity tag, as well as expressing specific chaperone proteins, is necessary to produce nAChRs in high-yield protein producing human cell lines. We will present data on purifying intact nAChRs within the membrane lipid environment using nanodiscs to allow for more detailed study of toxin-receptor interactions. Further characterization of the nAChR-neurotoxin interactions will inform development of anti-toxin therapeutics based on the structure of the nAChR itself.

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