Co-localization of the Lynx1-A prototoxin and α7 nicotinic acetylcholine receptors in human embryonic kidney 293 cells

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Lynx1-A is an endogenous Ly-6 family mammalian prototoxin with a particularly high affinity for the α7 homopentamer. Structural homology exists between Lynx1-A and the snake venom toxin alpha-bungarotoxin (α-BTX). Alpha-bungarotoxin binds irreversibly to skeletal nicotinic acetylcholine receptors (nAChRs), thereby inhibiting channel activation. Lynx1-A is proposed to have a similar function, acting as a cholinergic braking mechanism through its interactions at the binding pocket. While it has been suggested that it may also have modulatory role in receptor trafficking, subcellular co-localization has yet to be determined. Simultaneous visualization of Lynx1-A and α7 has been hindered by a lack of co-expressing stable cell lines and an absence of reliable antibodies. This project aims to elucidate the precise role of Lynx1-A through assessment of its subcellular co-localization with α7 nAChR subunits. We hypothesize Lynx1-A binds to the subunit in the intracellular space and alters receptor trafficking to the plasma membrane. Human gene transcripts for Lynx1-A and the α7 subunit were ligated into plasmids with differing epitope tags, transformed into high efficiency *E. coli*, isolated, and transfected into human embryonic kidney 293 (HEK 293) cells via calcium phosphate method. Use of antibodies directed against each epitope allowed for co-localization to be confirmed through immunofluorescence confocal microscopy. Though precise sites of subcellular co-localization could not be determined, substantial regions of overlapping expression lend support to the hypothesized modulatory interaction. Future works will include co-transfections with resistance to inhibitors of cholinesterase 3 (RIC3), a human protein that promotes α7 subunit trafficking. Further, assessments of receptor functionality, abundance, and localization will be examined through α-BTX live staining.