

Progress towards understanding the cellular biology and treatment of BoNT LC persistence.

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Certain serotypes of botulinum neurotoxin (BoNT) cause an extremely persistent flaccid paralysis while others have short-lived symptoms. When comparing those serotypes that cleave SNAP-25, clinical symptoms are particularly long-lived (3-4 months) with BoNT/A and BoNT /C whereas those resulting from BoNT/E exposure resolve more rapidly (<2 weeks) . BoNT/A persistence is caused by retention of a functional toxin protease light chain (LC) in the presynaptic terminal. To facilitate design of therapeutics that might shorten persistence, we have been investigating how BoNT LC trafficking, post-translational modifications, and cellular protein interactions influence persistence. Trafficking analysis of BoNT/A LC using fluorescence microscopy demonstrates that the first 9 amino acids of BoNT/A LC are critical for its membrane association. Furthermore, the di-leucine motif at position 425/426, as well as palmitoylation of the cysteine at residue 427, is important in lipid raft association but apparently is not required for membrane localization, *per se*. Modification of the di-leucine motif does not seem to alter stability of the toxin while progressive deletion of the carboxyl terminal domain of BoNT/A LC reduces stability. Analysis of post-translational modifications by our group and others has demonstrated phosphorylation, palmitoylation, ubiquitination, and potentially sumo modification of the BoNT/A LC, any of which might influence its relative stability and function. Our data indicate that, due to increased ubiquitin modification, the ubiquitin-proteasome system (UPS) degrades BoNT/E more rapidly than BoNT/A, possibly accounting for the difference in persistence. Yeast two hybrid analysis of BoNT/A LC interactors has revealed a number of “hits” that are related to ezrin/septin/rho regulation of actin cytoskeleton which may be involved in localization and stability of LC. We are currently testing these interactions with the goal of assessing their contribution to LC persistence.

We are developing therapeutic treatments that are designed to accelerate cellular degradation of BoNT LC by harnessing the cellular UPS using “designer” E3 ubiquitin ligases. To generate BoNT LC-directed designer ubiquitin ligases, we have utilized the RING domain of the cellular ubiquitin ligase, XIAP, and separately fused it to two different BoNT LC targeting domains. One LC binding domain is a non-cleavable form of SNAP25 (SNAP25nc) and the second is a high affinity anti-LC (VHH) domain derived from a single chain camelid antibody. Fusions of these LC targeting domains with other ubiquitin E3 ligase domains (eg., HECT, F-box) have also been prepared for testing . Initial experiments indicate that such designer ubiquitin ligases are effective in accelerating BoNT/A LC degradation.