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Cleaved Intracellular SNARE Peptides are Implicated in a Novel Cytotoxicity Mechanism of Botulinum Serotype C

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Introduction

Recent advances in intracellular protein delivery have enabled more in-depth analyses of cellular functions. A specialized family of SNARE proteases, known as Botulinum Neurotoxins, blocks neurotransmitter exocytosis, which leads to systemic toxicity caused by flaccid paralysis. These pharmaceutically valuable enzymes have also been helpful in the

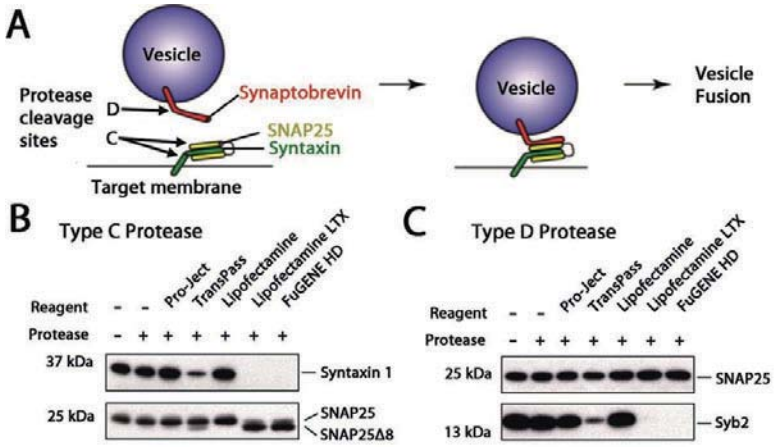

Fig. 1. (a) Schematic SNARE bundle formation. Western immunoblotting of syntaxin, SNAP25, and synaptobrevin for the type C protease; (b) and type D protease; (c) delivered with an array of transfection reagent.

study of SNARE functions. As can be seen in Figure 1A, SNARE bundle formation causes vesicle docking at the presynapse. Although these toxins are systemically toxic, no known cytotoxic effects have been reported with the curious exception of the Botulinum serotype C [1]. This enzyme cleaves intracellular SNAP25, as does serotype A and E, but also, exceptionally, cleaves Syntaxin 1. Using an array of lipid and polymer transfection reagents we were able to deliver different combinations of Botulinum holoenzymes into the normally unaffected, Neuro2A, SH-SY5Y, PC12, and Min6 cells to analyze the individual contribution of each SNARE protein and their cleaved peptide products.

Results and Discussion

Transfection reagents were able to abundantly and rapidly transduce SNARE proteases and peptides into cells [2]. Figure 1B shows the serotype C protease delivered into Neuro2A cells where it can cleave intracellular Syntaxin 1 and SNAP25 while Figure 1C shows the type D protease able to cleave synatobrevin. Using cell survival assays we also determined that the serotype C as well as the combination of type C and D caused profound cytotoxicity in the above-mentioned non-neuronal cell types that expands upon the observations seen on neuronal cells [1]. We observed that both apoptosis and necrosis mechanisms could be at play due to the appearance of morphonuclear abnormalities and increased propidium iodide staining respectively. Our results show that the freely released syntaxin and syntaxin plus

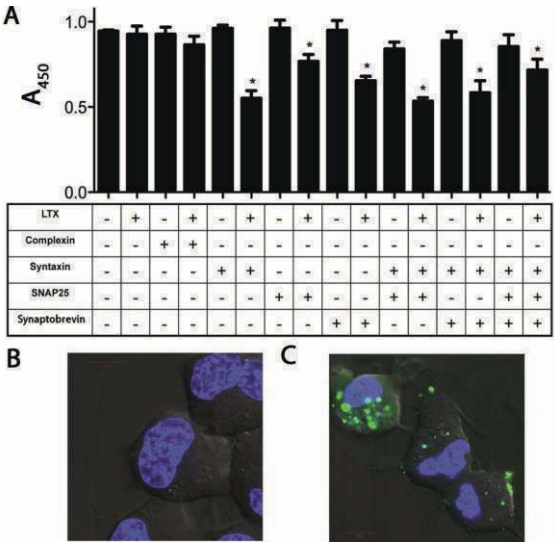

Fig. 2. (a) Cell survival assay of Neuro2A cells treated with SNARE peptide fragments. Neuro2A cells with FITC-syntaxin (45 aa) in the absence (b) or presence (c) of Lipofectamine LTX.

synaptobrevin peptides products, now separated from their transmembrane anchors and still able to form SNARE complexes (determined by GST-pull down experiments), could wreak havoc on the intracellular trafficking machinery. This mechanism was confirmed by the direct cellular penetration of the SNARE peptide fragments potentiated by the very same transfection reagents, Lipofectamine LTX. As can be seen in Figure 2A, SNARE peptides, mimicking the intracellular protease products, cause cytotoxic effects mirroring the loss of viability seen with the proteases. The viability of Neuro2A cells was strongly reduced in the presence of syntaxin as well as syntaxin plus synaptobrevin. The addition of all three SNARE subunits might partially rescue this cytotoxicity mechanism as they could stoichiometrically compete with each other preventing them from binding onto SNARE domains crucial for proper intracellular trafficking (Figure 2A last column). Figure 2B shows Neuro2A cells treated with the 45 aa fluorescent (FITC) syntaxin fragment while Figure 2C shows the same amount of FITC-syntaxin added in the presence of Lipofectamine LTX. A clear elevation of cell penetration can be observed. The freely diffusible syntaxin fragments, shown to cause cytotoxic effects in these tested cell types, were also shown to inhibit neurite outgrowth in differentiated PC12 cells. The treatment of *ex vivo* cortical cells with the holoenzymes and SNARE peptides showed a parallel cytotoxic effect and an observable Wallerian degeneration. Here we show for the first time that the type C's cytotoxic effect can also be observed in non-neuronal cells. Following our investigation we determine that the free floating, unanchored, syntaxin fragment is the exacerbating cytotoxic factor. Since syntaxin peptide fragments, compounded by synaptobrevin peptide fragments, cause cytotoxicity, we propose that the subsequent formation of erroneous SNARE complexes would have disastrous effects upon cell viability. These results open the door for a dual-acting pharmacological intervention that could simultaneously inhibit secretion while also destroying tumors cells. Our results thus open the door for translational trials of SNARE proteases and SNARE peptides as anti-neoplastic agents. This concomitant inhibition of exocytosis and cytotoxicity could also yield benefits for the targeted treatment of neuroendocrine disorders [3].

Acknowledgments

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References

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