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Basic Tetrapeptides as Potent Intracellular Inhibitors of Type A Botulinum Neurotoxin Protease Activity

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Botulinum neurotoxins (BoNT) are the most potent of all toxins. They cause flaccid muscle paralysis leading to death. They are also potential biothreat agents. A systematic investigation of various short peptide inhibitors of the BoNT protease domain with a 17-residue peptide substrate led to arginine-arginine-glycine-cysteine (RRGC) having a basic tetrapeptide structure as the most potent inhibitor. When assayed in the presence of dithiothreitol (DTT), the inhibitory effect was drastically reduced. Replacing the terminal cysteine with one hydrophobic residue eliminated the DTT effect but with two hydrophobic residues made the pentapeptide a poor inhibitor. Replacing the first arginine with cysteine or adding an additional cysteine at the N-terminus did not improve inhibition. When assessed using mouse brain lysates, the tetrapeptides also inhibited BoNT/A cleavage of the endogenous SNAP-25. The peptides penetrated the neuronal cell lines, N2A and M17, without adversely affecting metabolic functions as measured by ATP production, and P-38 phosphorylation. Biological activity of the peptides persisted within cultured chick motor neurons and rat cerebellar neurons for more than 40h, and inhibited BoNT/A protease action inside the neurons in a dose- and time-dependent fashion. Our results define a tetrapetide as the smallest peptide inhibitor in the backdrop of a large substrate protein of 200+ amino acids having multiple interaction regions with its cognate enzyme. The inhibitors should also be valuable candidates for drug development.

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