Double anchorage to the membrane and intact interchain disulfide bond are required for the low-pH-induced entry of tetanus and botulinum neurotoxins into neurons

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Purpose of study: To study the membrane translocation of the clostridial neurotoxins in neurons.

Methods used: We have developed a protocol based on an experiment first performed with diphtheria toxin. Conditions were found that by-pass the SV endocytosis step and induce the entry of the L chain directly from the plasma membrane. The neurotoxin, bound to the plasma membrane in the cold, was exposed to a warm, low pH extracellular medium, and the entry of the L chain was monitored by measuring its specific metalloprotease activity with a ratiometric method.

Summary of results: We found that the neurotoxin has to be bound to the membrane via at least two anchorage sites in order for a productive low-pH-induced structural change to take place. In addition, this process can only occur if the single inter-chain disulfide bond is intact. The pH dependence of the conformational change of tetanus neurotoxin and botulinum neurotoxins B, C and D is similar and take places in the same slightly acidic range, which comprises that present inside synaptic vesicles. Furthermore, using PROPKA3.0 software, we found that tetanus and botulinum neurotoxins share a pool of conserved acid residues that are predicted to be protonated in the pH range 4.5-6.

Conclusions: Tetanus and botulinum B, C and D neurotoxins undergo to a conformational change in a very similar pH range (4.5-6), which leads to light chain internalization. These neurotoxins share a pool of acidic residues which could be involved in the initial steps of the pH-dependent conformational change.

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Recovery and detection of botulinum toxin type A from drinking water

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Purpose of study: Botulinum neurotoxin (BoNT) represents a possible biothreat agent for drinking water systems. However, a standard method for monitoring drinking water potentially contaminated with BoNT is not currently available to response laboratories.

Methods used: Both the botulinum toxin ELISA and Endopep-MS (a mass spectrometric-based endopeptidase method) were adapted to quantify complex BoNT/A in deionized water and in chlorine-demand-free (CDF) water.

Summary of results: The method detection limit (MDL) of the ELISA and the MS methods in CDF water was 500 and 50 pg/ml of BoNT/A complex toxin, respectively. While adapting these methods to municipal tap water samples, residual chlorine in such samples was neutralized with 50 mg/L sodium thiosulfate prior to toxin spiking because free chlorine rapidly (<5 minutes) inactivates BoNT. Prior to testing by either ELISA or MS, municipal tap water samples from 5 U.S. states having distinct water quality properties were processed using ultrafiltration (UF) to concentrate the toxin by up to 200-fold. In order to evaluate the efficiency of toxin concentration using UF, 100 L tap water samples were spiked with 5 ug BoNT/A, which represents a final concentration equivalent to the MDL of the Endopep-MS method (i.e., 50 pg/ml). Recovery efficiencies of the UF method for BoNT/A complex, quantified by ELISA, ranged from 11%-36%, while efficiencies quantified by MS ranged from 26%-55%.

Conclusions: Both the botulinum toxin ELISA and the Endopep-MS methods were effective for the quantification of BoNT in municipal tap water samples of varying properties. Additionally, UF was capable of significantly increasing the BoNT/A concentration in tap water samples for analysis by either ELISA or Endopep-MS.

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Simultaneous detection and differentiation of botulinum toxin serotypes A and B by internally-quenched fluorogenic peptides

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Purpose of study: We sought to use a sensitive, non-invasive enzymatic activity assay for simultaneously detecting and distinguishing between active botulinum toxin (BoNT) serotypes A and B in one reaction and sample.

Methods used: To develop this assay, we used internally quenched fluorogenic peptides corresponding to SNAP-25 labeled with the fluorogenic dye FITC/DABCYL for BoNT/A and to VAMP 2 labeled with the fluorogenic dye o-Abz/Dnp for BoNT/B. Because each peptide is labeled with different fluorophores, we were able to distinguish between these two toxins. To reduce interference from the food matrix, we utilized immunomagnetic beads to capture, concentrate and remove the toxin from food.

Summary of results: This study reports that conventional milk pasteurization inactivated BoNT serotype A.