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Association of botulinum neurotoxins with synaptic vesicle protein complexes

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ABSTRACT

Botulinum neurotoxins (BoNTs) elicit flaccid paralysis by cleaving SNARE proteins within peripheral neurons. BoNTs are classified into seven serotypes, termed A–G, based on antibody cross-neutralization. Clostridia produce BoNTs as single-chain toxins that are cleaved into a di-chain protein that comprises an N-terminal zinc metalloprotease domain that is linked by a disulfide bond to the C-terminal translocation/receptor-binding domain. BoNT/A and BoNT/B utilize synaptic vesicle protein 2 (SV2) and synaptotagmin, respectively, as receptors for entry into neurons. Using affinity chromatography, BoNT/A and BoNT/B were found to bind a synaptic vesicle protein complex in CHAPS extracts of synaptic vesicles. Mass spectroscopy identified synaptic vesicle protein 2, synaptotagmin I, synaptophysin, vesicle-associated membrane protein 2, and the vacuolar ATPase-proton pump as components of the BoNT–synaptic vesicle protein complex. BoNT/A and BoNT/B possessed unique density-gradient profiles when bound to synaptic vesicle protein complexes. The identification of BoNT/A and BoNT/B bound to synaptic vesicle protein complexes provides insight into the interactions of BoNT and neuronal receptors.

The botulinum neurotoxins (BoNTs) are the most potent protein toxins for humans and have been designated by the CDC as category A agents (Shapiro et al., 1997; Sobel, 2005). BoNTs elicit flaccid paralysis by blocking acetylcholine release at the neuromuscular junction in peripheral α-motor neurons through the cleavage of SNARE proteins. In contrast, tetanus toxin elicits spastic paralysis by blocking glycine release in the central nervous system through the cleavage of the SNARE protein, vesicle-associated membrane protein 2 (VAMP-2). Recent studies have identified the neuronal receptors for these neurotoxins; our studies have identified a neuronal vesicle protein complex that BoNT/A and BoNT/B bind with high affinity.

1. Structure–function properties of the botulinum neurotoxins

BoNTs are classified into seven serotypes (designated A–G) based upon the neutralizing capacity of α-sera, where neutralizing α-sera to BoNT/A does not neutralize BoNT serotypes B–G (Smith et al., 2005). BoNTs serotypes differ up to 70% at the primary amino acid level and up to 32% within a serotype (Lacy and Stevens, 1999; Smith et al., 2005). Clostridia produce BoNTs as single-chain proteins that are cleaved to di-chain proteins that are linked through a disulfide bond. BoNTs are AB toxins that comprise a catalytic light-chain (LC, ~50 kDa) and a heavy chain (HC, ~100 kDa) (DasGupta and Dekleva, 1990). LC comprises a zinc metalloprotease domain that cleaves SNARE proteins; serotypes A, C, and E cleave SNAP25, serotypes B, D, F, G, and tetanus toxin cleave VAMP-2, and serotype C also cleaves syntaxin 1a (Blasi et al., 1993a,b; Schiavo et al., 1992; Schiavo et al., 1994, 1993). HC comprises two

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domains: an N-terminal translocation domain (HCT, ~50 kDa) and a C-terminal receptor-binding domain (HCR, ~50 kDa) (Lomneth et al., 1993). The HCR domain is further divided into two smaller domains termed HCR_N and HCR_C (Fig. 1a). HCR_N does not have a functional activity, but may be involved in aligning the HCT for optimal translocation of the LC into the host cell, while HCR_C mediates the binding of BoNTs to host receptors (Lalli et al., 1999; Rummel et al., 2004). Lacy and Stevens solved the crystal structure of BoNT/A (Lacy et al., 1998) (Fig. 1b), which provided the first insight into the structure–function organization of this family of toxins and provided a foundation to conduct reverse genetic studies on toxin action. The three domains of BoNT are organized into distinct structures that account for the compartmentalization of protein function. One of the unusual aspects of the holo-BoNT structure is that the N-terminal region of the HC wraps around the LC in a manner that is analogous to the mechanism that is utilized by the LC to bind SNARE proteins (Breidenbach and Brunger, 2004; Chen and Barbieri, 2007). The extended substrate binding regions of the BoNTs explain how these proteases efficiently recognize and cleave their coiled SNARE substrates.

2. Botulinum neurotoxin–host receptor interactions

BoNTs preferentially intoxicate neurons at the presynaptic membrane of α-motor neurons (Johnson and Bradshaw, 2001). Recent studies have begun to unravel the steps involved in the binding and entry of the BoNTs into neurons. BoNTs intoxicate neurons through a multi-step mechanism that involves neuronal cell binding, vesicle internalization by receptor-mediated endocytosis and/or synaptic vesicle uptake, and LC translocates across membranes in acidified endosomes to target and cleave the SNARE substrate (Fig. 2). In vivo stability and resistance to degradation of internalized LC contribute to the longevity of BoNT morbidity. The current model for BoNT binding to peripheral neurons follows the model proposed by Montecucco et al. (2004) where BoNTs bind neurons through dual-receptor interactions that involve an initial binding to ganglioside, which increases the BoNT membrane concentration with subsequent binding to a protein receptor(s). Kozaki et al. (1998) observed that BoNT/B bound to lipid vesicles containing synaptotagmin and GT1b or GD1a and that a monoclonal antibody against GT1b inhibited BoNT/B binding to brain synaptosomes and inhibited the action of BoNT/B on synaptic transmission of neurons (Ochanda et al., 1986). The nature of the protein component of the BoNT/B receptor was determined by Dong et al. (2003) who observed that entry of BoNT/B into PC12 cells and motor neurons was activity dependent. Subsequent studies showed that peptide fragments of the luminal loops of synaptotagmin functioned as physiological BoNT/B receptors. Using a similar approach, Dong et al. (2006) and Mahrhold et al. (2006) reported that the luminal loop of synaptic vesicle protein 2 (SV2) was the receptor for BoNT/A. The BoNT–receptor complex enters neurons by receptor-mediated endocytosis. Upon acidification of the early endosome by the proton pump, the HCT domain of BoNT inserts into the endosome membrane and forms a pore that allows the translocation of the LC into the cytosol. Koriazova and Montal (2003) detected currents in BoNT/A and HC/A-treated bi-layers and cleavage of BoNT substrate in the trans-compartment, which implicated a role for the HCT in LC translocation across the endosome membrane.

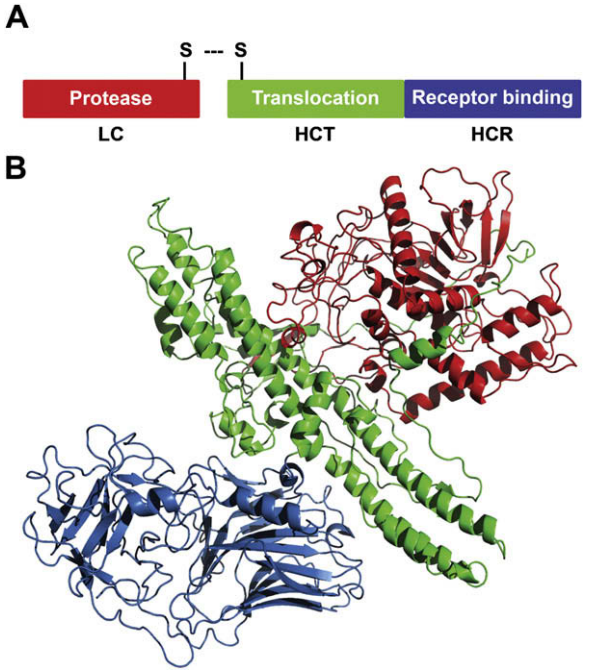

Fig. 1. Structure–function properties of the botulinum neurotoxins. (A) Ribbon diagram of BoNT/A (pdb: 3bta). (B) Linear diagram of the di-chain activated BoNT molecule consisting of a light (LC) and heavy chain (HC) bound by a single disulfide bond. HC is divided into an N-terminal translocation domain (HCT) and a C-terminal receptor-binding domain (HCR).

3. Synaptic vesicle protein complexes

Neuronal synaptic transmission is initiated when an action potential induces the elevation of intracellular Ca^{2+}

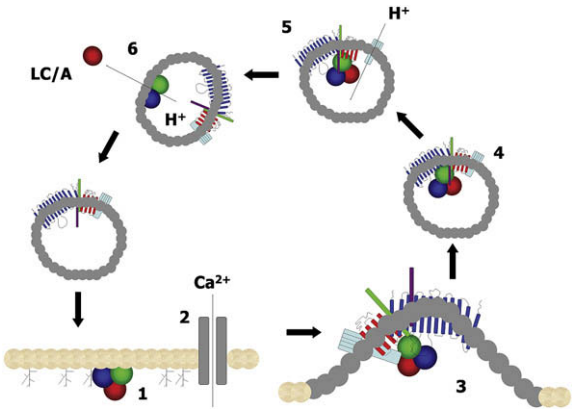

Fig. 2. Model for binding and entry of BoNTs at the neuromuscular junction BoNT/A associates with the presynaptic membrane of α-motor neurons through interactions with oligosaccharides such as ganglioside G_{T1b} (1). Calcium influx stimulates synaptic vesicle membrane fusion (2). BoNT/A interacts with the exposed receptor complex, stimulating synaptic vesicle recycling (3). Recycled synaptic vesicles are acidified through the activity of the v-ATPase (4). Vesicle acidification drives the translocation of the BoNT/A light chain (LC/A) into the cytosol (5) completing the cycle (6).