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Receptor and substrate interactions of clostridial neurotoxins

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ABSTRACT

The high potency of clostridial neurotoxins relies predominantly on their neurospecific binding and specific hydrolysis of SNARE proteins. Their multi-step mode of mechanism can be ascribed to their multi-domain three-dimensional structure. The C-terminal H_{CC}-domain interacts subsequently with complex polysialo-gangliosides such as GT1b and a synaptic vesicle protein receptor via two neighbouring binding sites, resulting in highly specific uptake of the neurotoxins at synapses of cholinergic motoneurons. After its translocation the enzymatically active light chain specifically hydrolyses specific SNARE proteins, preventing SNARE complex assembly and thereby blocking exocytosis of neurotransmitter.

1. Introduction

The family of clostridial neurotoxins (CNTs) consists of tetanus neurotoxin (TeNT) and the seven botulinum neurotoxin serotypes (BoNT/A-G), and represents the most toxic agents known. The median lethal dose is below 1 ng per kg of body weight (Gill, 1982). The disease tetanus is caused by germination of Gram-positive, anaerobic spore-forming *Clostridium tetani* in infected tissue lesions, thereby producing and releasing TeNT into the blood stream. In contrast, botulism is evoked by ingestion of acid resistant BoNT progenitor toxins, generated by various strains of *C. botulinum*, *C. butyricum* and *C. barati*, and subsequent transcytosis of this complex or the released BoNT through the intestinal epithelial barrier (Bigalke and Shoer, 2000). The CNTs reach the motoneurons via circulation and specifically bind to unmyelinated areas of nerve terminals (Dolly et al., 1984). Here, BoNTs inhibit acetylcholine release followed by flaccid paralysis while TeNT is transported retrogradely to inhibitory neurons and blocks release of glycine or γ-aminobutyric acid which results in spastic paralysis.

The crystal structures of the BoNT/A, B and E holotoxins (Lacy et al., 1998; Swaminathan and Eswaramoorthy, 2000, Kumaran et al., 2009) revealed that most likely all CNTs are composed of four functionally independent domains that perform individual tasks in the multi-step intoxication process (Fig. 1). All CNTs are produced as ~150 kDa single chain (sc) proteins. They are post-translationally proteolysed into a ~100 kDa heavy chain (HC) and a ~50 kDa light chain (LC). Both chains remain associated by a single disulphide bridge, non-covalent interactions and an HC derived peptide loop wrapping around the LC. The HCs are responsible for neurospecific binding, uptake and translocation of the LCs into the cytosol. Following cell attachment, internalisation via receptor-mediated endocytosis brings the BoNTs into the synaptic vesicles. Here, the acidic environment eliminates repulsive electrostatic interactions between the largely α-helical amino-terminal half of the

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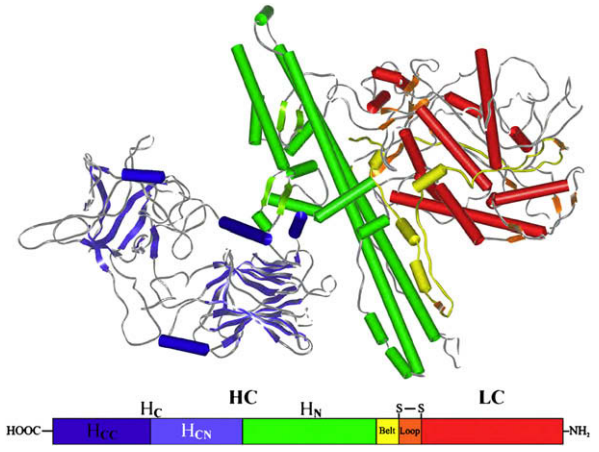

Fig. 1. Schematic representation of the four-domain structure of the single-chain 150 kDa clostridial neurotoxins (bottom) and the corresponding crystal structure of BoNT/B (top; modified from 1EPW.pdb). The clostridial neurotoxins are post-translationally proteolysed within the loop segment into LC and HC which remain linked together covalently by a disulphide bridge and non-covalently by a "belt" wrapping around the LC. The C-terminal half of the H_C-fragment, the H_{CC}-domain is responsible for neurospecific binding to complex polysialo-gangliosides and a synaptic vesicle protein receptor, and subsequent uptake, the H_N-domain translocates the LC into the cytosol where the latter acts as metalloprotease.

HC, the H_N-domain and the membrane, allowing its penetration into the membrane, without triggering detectable structural changes (Galloux et al., 2008). At the same time the LC is partially unfolded (Koriazova and Montal, 2003). Translocation of LC by HC can be observed in real time as an increase of channel conductance. The HC channel is occluded by the LC during transit, then unoccluded after completion of translocation and release of LC (Fischer and Montal, 2007b). Upon reduction of the disulphide bond, the LC functions as a zinc dependent endopeptidase in the cytosol (Fischer and Montal, 2007a; Schiavo et al., 1990).

2. Gangliosides as receptors for CNTs

The specific binding to peripheral nerve endings at the neuromuscular junction solely involves the 50 kDa C-terminal half of the HC, the H_C-fragment (Evinger and Erichsen, 1986; Fishman and Carrigan, 1987; Lalli et al., 1999; Simpson, 1984a,b, 1985) and complex polysialo-gangliosides, glycosphingolipids that are found particularly in membranes of neuronal cells (Simpson and Rapport, 1971; van Heyningen and Miller, 1961). The interaction of gangliosides with CNTs was investigated for TeNT and several serotypes of BoNTs in extensive studies (Halpern and Neale, 1995; Yowler and Schengrund, 2004). These studies revealed that the disialo-carbohydrate structure as found in GD1b is essential for the binding of most of the CNTs. Furthermore, TeNT, BoNT/A, B, C, E, and F have affinities in the upper nM range in various *in vitro* binding assays with immobilised polysialo-gangliosides, whilst CNTs have much higher affinity ($K_D = 1.2$ nM) to synaptosomes that are similar to neuronal tissue. At the cellular level, the cleavage of sialic acid residues by neuraminidase treatment of cultured cells isolated from spinal cord (Bigalke et al., 1986) and adrenergic chromaffin cells (Marxen et al., 1989) reduced BoNT/A potency as well as TeNT action (Critchley et al., 1986). Conversely, bovine chromaffin cells lacking complex polysialo-gangliosides were rendered sensitive to TeNT and BoNT/A by incubation with gangliosides (Marxen and Bigalke, 1989; Marxen et al., 1991). In addition, a monoclonal antibody to GT1b antagonised the action of BoNT/A on rat superior cervical ganglion neurons (Kozaki et al., 1998). The inhibition of ganglioside biosynthesis with fumonisin in primary spinal cord neurons and with D,L-threo-1-phenyl-2-hexadecanoylamino-3-morpholino-propanol–HCl (PPMP) in the neuroblastoma cell line Neuro2a resulted in insensitivity to TeNT and BoNT/A, respectively (Williamson et al., 1999; Yowler et al., 2002). Employing a genomic approach, mice, deficient in *NAcGal-transferase* thus only expressing Lac-Cer, GM3 and GD3, resisted treatment with TeNT and BoNT/A and B (Bullens et al., 2002; Kitamura et al., 1999) whereas *GD3-synthase* knock-out mice expressing only Lac-Cer, GM3, GM2, GM1 and GD1a are solely resistant to TeNT, but kept their sensitivity towards BoNT/A, B and E (Kitamura et al., 2005). A combination of both gene knock-outs resulted in GM3-only mice which display *inter alia* high resistance towards BoNT/B and G (Rummel et al., 2007). Also, *GM3-synthase* knock-out mice theoretically expressing only Lac-Cer are insensitive to BoNT/C1 (Tsukamoto et al., 2005). Hence, complex polysialo-gangliosides such as GD1a, GD1b and GT1b mediate the first cell contact of CNT and play an important role in their specific binding to neuronal cells.

3. A protein is the second receptor for CNTs

The discrepancy in affinity between binding of CNTs to isolated gangliosides and neuronal tissue prompted predictions of a second receptor component. The protease-sensitive binding of BoNT/A and TeNT to rat brain synaptosomes (Dolly et al., 1982; Kitamura, 1976; Lazarovici and Yavin, 1986; Pierce et al., 1986) resulted in a dual receptor model. First, polysialo-gangliosides were considered to accumulate CNTs on the plasma membrane surface. Then, CNTs would simply stay on the surface until binding is accomplished to their thinly distributed protein receptor(s) or move laterally within the membrane while still bound to low affinity receptors thereby increasing the chance of contact with the protein receptor. Simultaneous interaction with ganglioside and protein receptor would then be considered as high affinity binding and set the stage for the subsequent specific step of endocytosis (Montecucco, 1986; Niemann et al., 1991).

Several studies demonstrated accelerated uptake of TeNT (Simpson, 1985) and BoNT/A (Black and Dolly, 1986) upon electrical stimulation into hemidiaphragm preparations as well as of BoNT/A and E upon K^+ stimulation into spinal cord neurons (Keller et al., 2004). As a consequence, increased nerve stimulation resulted in an earlier onset of neurotransmitter blockade upon application of BoNT/A (Hughes and Whaler, 1962; Simpson, 1980) and TeNT (Schmitt et al., 1981). As nerve stimulation causes increased rates of exo- and endocytosis of synaptic vesicles, one can hypothesise that synaptic vesicle proteins, which, upon neurotransmitter release, become temporarily exposed on