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Review

Botulinum neurotoxins: Mechanism of action

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ABSTRACT

Botulinum neurotoxins are used clinically for conditions characterized by hyperexcitability of peripheral nerve terminals and hypersecretory syndromes. These neurotoxins are synthesized as precursor proteins with low activity, but their effects are mediated by the active form of the neurotoxin through a multistep mechanism. Following a high-affinity interaction with a protein receptor and polysialogangliosides on the synaptic membrane, botulinum neurotoxins enter the neuron and causes a sustained inhibition of synaptic transmission. The active neurotoxin is part of a high-molecular-weight complex that protects the neurotoxin from proteolytic degradation. Although complexing proteins do not affect diffusion of therapeutic neurotoxin, they may lead to the development of neutralizing antibodies that block responsiveness to it. Nerve terminal intoxication is reversible and its duration varies for different BoNT serotypes. Although it was previously assumed that botulinum neurotoxins exert effects only on the peripheral synapses, such as the neuromuscular junction, there is now substantial evidence that these neurotoxins affect neurotransmission at distal central nervous system sites as well.

1. Introduction

Botulinum neurotoxins (BoNTs) and tetanus neurotoxin (TeNT) are metalloproteases that act on nerve terminals to prevent exocytosis (Dressler and Benecke, 2007; Montal, 2010). Both BoNTs and TeNT bind with extreme specificity to neurons and are internalized at the neuromuscular junction and other nerve terminals. TeNT acts mainly at inhibitory synapses located in the spinal cord and other sites in the central nervous system (CNS), whereas BoNTs act by inhibiting neurotransmitter release mainly at neuromuscular junctions (Rossetto and Montecucco, 2008).

BoNT is produced by *Clostridium botulinum* and other related *Clostridium* species (e.g., *barati*, *butyricum*). There are seven serologically distinct BoNT isoforms (denoted A–G) (Lacy and Stevens, 1999). BoNT are encoded by *bont* genes (~3880 bp), which exhibit 34%–97% sequence similarity for the seven BoNT serotypes (Poulain, 2008). Numerous subtypes have been recently identified for at least six of the seven serotypes; seven subtypes of BoNT-A are currently recognized, the main being A1, A2, A3, A4, and A5 (Arndt et al., 2006; Hill et al., 2007, 2009; Peck, 2009). Four of the BoNT serotypes (A, B, E, and F) have caused outbreaks of the human neuroparalytic disease botulism (Arnon et al., 2001).

BoNTs are used clinically for the treatment of several disorders characterized by hyperexcitability of peripheral nerve terminals and hypersecretory syndromes. BoNT preparations are a group of highly potent drugs with a very specific mechanism of action (Dressler and Benecke, 2007;

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Montal, 2010). Depending on the target tissue, BoNTs can block the cholinergic neuromuscular or cholinergic autonomic innervation of exocrine glands and smooth muscles. Additional effects can be demonstrated on the muscle spindle organ. Indirect effects on the CNS have been documented, and are currently under study. Therapeutic BoNT-A preparations include onabotulinumtoxinA (A/Ona), abobotulinumtoxinA, and incobotulinumtoxinA (A/Inco); BoNT-B is available as rimabotulinumtoxinB (Dressler and Benecke, 2007).

2. Botulinum neurotoxin structure

Each BoNT serotype is synthesized as a single polypeptide chain with a molecular mass of about 150 kDa (Montal, 2010; Rossetto and Montecucco, 2008). This precursor protein displays intrinsic low activity. The 150 kDa precursor protein is cleaved either by clostridial or tissue proteases into a 50 kDa light chain (LC) and a 100 kDa heavy chain (HC) (Rossetto and Montecucco, 2008). The LC and HC are linked by an essential interchain disulfide bridge and a poorly structured protein segment, called the belt, which extends from the HC and wraps around the LC (Montal, 2010; Rossetto and Montecucco, 2008). The active, mature toxin consists of three modules: an N-terminal LC zinc-protease, the HC that encompasses the N-terminal ≈50 kDa translocation domain (H_N), and the C-terminal ≈50 kDa receptor-binding domain (H_C) (Fig. 1) (Kumaran et al., 2009; Lacy and Stevens, 1999; Lacy et al., 1998; Montal, 2010; Swaminathan and Eswaramoorthy, 2000). The H_C comprises two subdomains: a β-sheet jelly roll fold, denoted H_{CN}, and a β-tree foil fold subdomain located at the C-terminus of HC, known as H_{CC} (Montal, 2010).

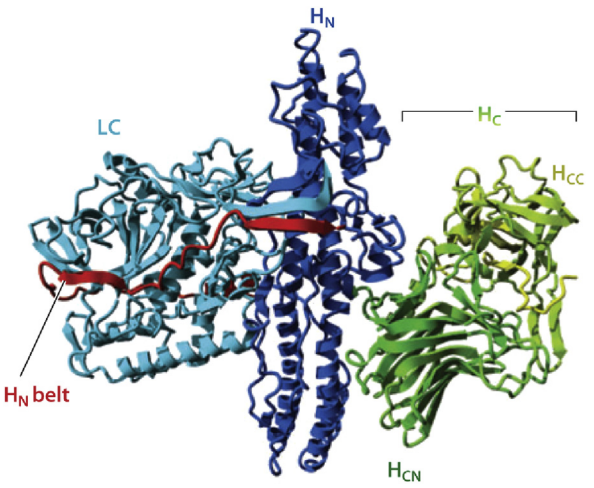

Fig. 1. Structure of BoNT-A. The Cα backbone is represented as ribbons, with the LC in cyan (blue-green), H_N in dark blue, and H_C in a green-to-yellow gradient highlighting the H_{CN} and H_{CC} subdomains. The H_N belt is depicted in red. From Montal et al. (Montal, 2010). BoNT-A = botulinum neurotoxin A, HC = heavy chain, H_C = C-terminal ≈50 kDa receptor-binding domain, H_{CC} = 25-kDa C-terminal half of H_C, H_{CN} = β-sheet jelly roll fold, H_N = N-terminal ≈50 kDa translocation domain, LC = light chain. Adapted with permission of Annual Reviews, Inc. from Botulinum neurotoxin: a marvel of protein design, Montal M., 79, 2010. Permission conveyed through Copyright Clearance Center, Inc.

3. Events at the neuromuscular junction

BoNTs have a very high-affinity and specificity for their target cells and require two different co-receptors located on the neuronal cell surface (Montal, 2010; Rossetto and Montecucco, 2008). The effects of BoNTs commence by the binding of the neurotoxin at the nerve terminal via the H_C to surface receptors (Rossetto and Montecucco, 2008). For the majority of BoNT serotypes, such receptors are composed of a polysialoganglioside and a synaptic vesicle protein that is transiently exposed on the surface of nerve terminals (Schiavo, 2006).

Different serotypes have different protein receptors: SV2 (isoforms A-C) for BoNT-A, BoNT-E, and BoNT-F, and synaptotagmin (Syt) I and II for BoNT-B and BoNT-G (Montal, 2010). However, the protein receptor(s) for BoNT-C and BoNT-D remain a subject of debate. Although Peng and colleagues recently showed that BoNT-D also binds to SV2 (Peng et al., 2011), growing evidence suggests that for BoNT-C and BoNT-D, binding to a protein receptor may have been replaced by additional interactions with lipids. However, the precise nature of the lipid moieties involved in this binding is presently unclear (Bercsenyi et al., 2013). In the case of BoNT-A, BoNT-E, and BoNT-F, the H_C receptor-binding domain is responsible for binding to the oligosaccharide portion of polysialogangliosides and several isoforms of the synaptic vesicle protein SV2. Binding to SV2 occurs at the level of a conserved loop that is located within the lumen of synaptic vesicles. This loop is transiently exposed on the surface of nerve terminals upon synaptic vesicle exocytosis (Karalewitz et al., 2010; Strotmeier et al., 2010; Tsukamoto et al., 2005; Zhang et al., 2010).

BoNTs exert their neurotoxic effect by a multistep mechanism: binding, internalization, intracellular traffic, membrane translocation, and proteolytic degradation of specific intracellular targets (Montal, 2010; Rossetto and Montecucco, 2008). Binding of BoNTs to the neuromuscular junction involves the tight association between BoNTs with complex polysialogangliosides known to be enriched in neurons. Among these, disialo (GD1b)- and trisialo- (GT1b) gangliosides exhibit BoNT binding affinities in the nM range and thus establish an initial anchorage to the neuronal membrane (Fig. 2). Overall, BoNT serotypes A, B, C, and F bind GT1b, GD1b, and GD1a. BoNT-E binds GT1b and GD1a, whereas BoNT-G recognizes all polysialogangliosides with approximately similar affinity. The polysialoganglioside-binding pocket is confined to H_{CC} (Montal, 2010). In contrast, the role of H_{CN} is less clear; it has been linked to the recruitment of BoNT-A to sphingomyelin-enriched lipid microdomains via binding to phosphatidylinositides (Muraro et al., 2009).

BoNTs enter cells mainly by exploiting synaptic vesicle recycling (Rossetto and Montecucco, 2008; Simpson, 2004). The HC is inserted into the synaptic vesicle membrane and forms a transmembrane protein-conducting channel that translocates the LC to the cytosol, where it acts (Montal, 2010).