Wrinkle Release: The Entry Mechanism of Botulinum Neurotoxin

Cudahy SMART Team: Sara Kutcher, Jazmin Jones, Abby Jones, Katherine MacDonald,
Katya Tolbert, Alex Romfoe, Amber Perkins, Paige Broeckel, Angelica Prado and Virginia Lachenschmidt
Mentors: Joseph Barbieri, PhD. and Andrew Karalewitz, Ph.D., Microbiology, Medical College of Wisconsin
Advisor: Dan Koslakiewicz

Abstract

Botulism is a potentially fatal disease or therapeutic for muscular disorders, which results from intoxication of cells by the protein botulinum neurotoxin (BoNT). BoNT, produced by the bacteria *Clostridium botulinum,* paralyzes humans through inhibition of neuromuscular synaptic transmission. BoNT cleaves the Soluble NSF Attachment Protein Receptor (SNARE) proteins responsible for guiding synaptic vesicles carrying neurotransmitters for muscle stimulation to the neural plasma membrane, resulting in the muscle relaxation. BoNTs must first gain entry to the neuron using a ganglioside binding domain (GBP) that recognizes a specified ganglioside, a complex of carbohydrates and sialic acid within the neural plasma membrane. The sialic acid region of the ganglioside binds with specific residues on the BoNT GBP2: Tyr1115, Ser1275, Ile1240, Tyr1243, and Ser1242, modeled by the Cudahy SMART (Students Modeling A Research Topic) Team using 3D printing technology. After binding, the toxin is able to access a vesicle, crossing into the cytoplasm of the neuron, where the light chain of the toxin can cleave the SNARE proteins, causing a loss of muscular function due to lack of neural stimulation. In the case of a systemic BoNT intoxication, lack of muscle function can lead to respiratory failure but when used as a therapeutic BoNT relaxes specific muscles. While the action of the neurotoxin BoNT is well understood inside the neuron, the mechanism of entry is not well known. Understanding how BoNT recognizes and enters the neuron allows researchers to develop better treatments for infections and improved therapies to treat spastic muscle disorders.

What is botulism?

http://www.chinaaseansps.com/show.aspx?id=748&cid=215

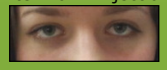
It is a disease caused by the bacterium *Clostridium botulinum.* Neurons become intoxicated by BoNT, preventing muscle stimulation, resulting in paralysis. In some instances, the bacterial infection and subsequent neuron intoxication can be fatal. First recorded case of botulism occurred in 1735 and was associated with sausage.

Reasons for Use

While it is often associated with disease, BoNT has the potential for beneficial use. As a therapeutic, BoNT can be used to alleviate symptoms of blepharospasm, a disorder in which spasmodic contractions of orbicularis oculi muscles occur, preventing the eyes from fully opening. Symptoms include dry eyes, increased blinking, and blindness. Treatment with BoNT injections to paralyzes the spasmodic muscles allowing normal blinking to return, restoring sight.

Before BoNT injection

After BoNT injection

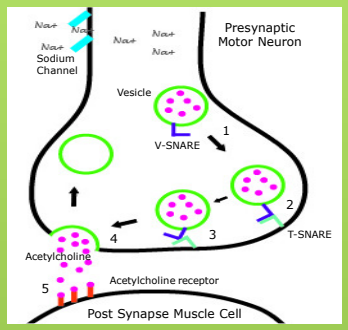


BoNT is also found as an ingredient for cosmetic Botox injections. In low doses, it is injected under the skin, causing temporary paralysis of superficial muscles, removing the appearance of wrinkles.

http://www.columbiaderm.com/medical-spa/smooth-wrinkles/

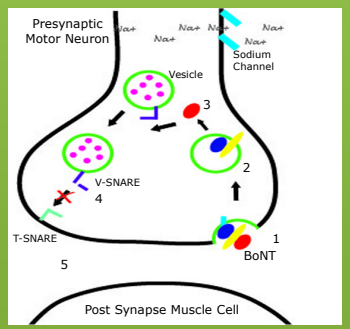
The action of BoNT in neurons

Normal Presynaptic Vesicle Fusion



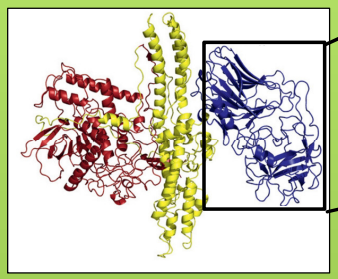
1. Vesicle in presynaptic neuron containing neurotransmitters is signaled to fuse with the plasma membrane.
2. Docking is achieved through binding of V-SNARE protein on the vesicle and T-SNARE proteins on the membrane.
3. Fusion is accomplished when calcium enters the presynaptic neuron terminal.
4. When the vesicle fuses with the membrane, the neurotransmitter, acetylcholine, is released.
5. The binding of acetylcholine to the receptors on the post-synaptic membrane found in muscle cells results in a muscle contraction.

BoNT Influenced Vesicle Fusion

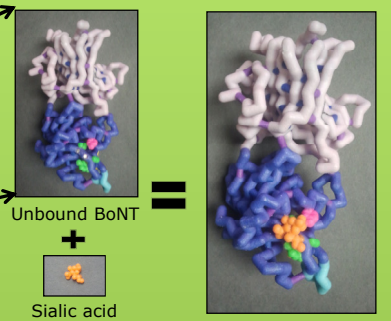


1. BoNT binds to sialic acid portion of ganglioside.
2. Binding causes the BoNT to be taken up by the presynaptic neuron through a vesicle.
3. Once inside, the BoNT is disassembled and a portion is released into the cytosol.
4. This portion cleaves either the V-SNARE or the T-SNARE, prohibiting the neurotransmitter vesicle from fusing with the plasma membrane.
5. No neurotransmitters were released, resulting in no muscle stimulation, causing paralysis.

The Structure of BoNT



The Binding of BoNT



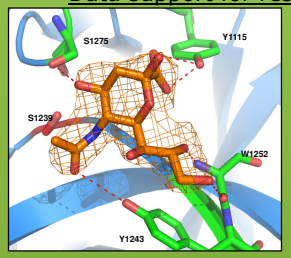
Domains of BoNT

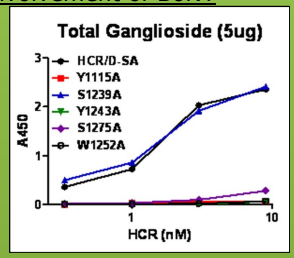
The Receptor Domain (blue) allows BoNT to bind to a sialic acid (orange) portion of a ganglioside found on motor neurons using key residues (Tyr1115, Ser1275, Ile1240, Tyr1243, and Ser1242) allowing for the attachment of the entire protein to gain entry into the neuron via a vesicle. Without entry into the neuron, BoNT will not cause muscle paralysis.

Once inside the neuron, the translocation domain (yellow), pierces the vesicle. This delivers the protease domain (red) to the cytosol. Once the protease is allowed into the cytosol, it is free to move to the SNARE protein.

The cleaving of the SNARE protein by the protease domain achieves muscle paralysis. None of this is possible it entry into the neuron is inhibited.

Data support for residue involvement of BoNT





Shown in green are residues that are actively involved in the binding of BoNT to sialic acid. Their involvement has been confirmed through experimentation in which each suspected residue was changed individually. The effectiveness of BoNT-ganglioside binding was assessed. Each of the green residues exhibited a reduced binding affinity for the ganglioside, indicating they are necessary for the binding of BoNT to the ganglioside even with an increase in BoNT concentration. Only S1239, showed no involvement in binding. Dashed lines represent hydrogen bonds between the HCR and the ganglioside.

Conclusions

The activity of the protease domain inside of the motor neuron is understood. However, if BoNT is not able to bind to the cell, this domain is useless. There is cause for studying the mechanism of BoNT entry into neurons. Confirmation of specific binding residues (Tyr1115, Ser1275, Ile1240, Tyr1243, and Ser1242) was necessary to this understanding. In knowing how BoNT enters the neuron, it is possible to develop a more effective delivery methods for therapeutics, reduce possible harmful reactions, and develop better treatments for BoNT intoxication before entry into a neuron.

References
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Kroken, A. R., Karalewitz, A. P.-A., Fu, Z., Baldwin, M. R., Kim, J.-J. P. and Barbieri, J. T. (2011), Unique ganglioside binding by botulinum neurotoxins C and D-SA. FEBS Journal. 278: 4486–4496.
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