Botulinum Neurotoxin B: The Biochemical Blade

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Abstract

Botulinum neurotoxins (BoNTs) are highly toxic proteins that cause the fatal neuroparalytic illness called botulism. BoNTs are produced by the anaerobic bacteria Clostridium botulinum. Although most prominently contracted from contaminated food, botulism can also be contracted from the soil, through the air, or from an open wound. BoNTs are AB toxins composed of three domains. The first domain, the A domain of BoNT, is the catalytic component, a Zinc-dependent protease. The second and third domain, or the translocation and receptor-binding domain respectively, comprise the B domain of the toxin, the binding component. Botulinum neurotoxin serotype B (BoNT/B) is a specific type of neurotoxin that binds to the neurons. The membrane of a neuron depolarizes which then stimulates the transport of calcium ions into the neuron. Proteins on the transmitter vesicle bind the calcium and the vesicles are then transported to the plasma membrane by SNARE proteins to release neurotransmitter acetylcholine. BoNT/B enters the lumen of the neurotransmitter vesicle and binds to the luminal loop of synaptotagmin, causing the loop to change from a coil to an alpha helix. This represents the high affinity binding of BoNT/B to neurons. Inside the neuron, the A domain of the toxin is translocated across the vesicle membrane by the translocation domain in a pH-dependent mechanism and cleaves the vesicle associated membrane protein (VAMP). This prevents neurotransmitter vesicles from fusing to the plasma membrane and inhibits further release of acetylcholine. Since acetylcholine is important in movement and memory, a lack of acetylcholine causes the nervous system to slow down and causes flaccid paralysis of the muscles, otherwise known as botulism.

Botulinum Neurotoxin Therapies

• First used as a therapeutic agent in the 1960’s
• Used to treat spastic muscle disorders such as dystonia (a movement disorder where the muscles contract involuntarily) and blepharospasms (a disability due to muscle spasms in eyelids which causes visual impairment, resulting in functional blindness)
• Treated by injecting minute dosages of the toxin directly to the muscles in the eyes, which will temporarily paralyze them by blocking the release of acetylcholine.

Inhalation Botulism

Symptoms of inhaling the neurotoxin consist of difficulty moving eyes, slurred speech, and severe muscle weakness. Other symptoms include sluggishly reactive pupils, dry mouths, and hyperventilation. In Picture A, patient is at rest; one can see his drooping eyelids, dilated pupils, and muscle symmetry. In Picture B, when asked to smile, the patient exhibits unsymmetrical muscle movement. The patient also demonstrates an absence of smile creases.

Ganglioside Binding Assay

Because BoNT/B binds simultaneously to the protein synaptotagmin as well as a lipid ganglioside, an experiment was designed to discover the amount of Hcr A1 (Heavy Chain Receptor subtype A) needed to reach the optimal level of ganglioside (Gt1b) binding. Bovine albumin was added to block all other receptors except the ganglioside; then Hcr A1 was added to each well of the assay incrementally. The data show that more concentrated amounts of Hcr A1 bind greater amounts of Gt1b, resulting in a higher absorbency. The 50% binding was observed at ~300 nM HCR A1. This knowledge helps scientists in further research towards the discovery of vaccines.

references


The Action of Botulinum Neurotoxin B

Normal Vesicle Function

•Synaptotagmin protein on ACh-filled vesicle detects incoming calcium
•Three SNARE proteins coil together, bringing the vesicle to the membrane
•Through exocytosis, ACh is released and the vesicle is then recycled back into the neuron.

Impaired Vesicle Function

•BoNT/B sits on exterior of membrane bound with high affinity
•When the luminal domain of the vesicle is exposed, BoNT/B binds to the synaptotagmin
•A signal transduction pathway is initiated and vesicle is recycled back into the neuron with the toxin
•The toxin’s ganglioside receptors also bind to the lumen of the vesicle
•Through a proton pump, H+ ions enter the vesicle, and the pH of the lumen decreases

Conclusion

The BONT/B ultimately prevents the release of acetylcholine from the axon terminal membrane, causing flaccid paralysis of the skeletal muscles. Knowing about this toxin and the way it binds allows us to develop therapies that can help cure people with spastic muscle disorders.

References