Clostridial Neurotoxins and Their Mechanisms

Introduction

In this document we will deal with the topic of Clostridial neurotoxins, their molecular structure and the mechanisms by which they function.

Clostridium

Clostridium is a genus of anaerobic gram positive rods. It contains multiple species of various importance, the most clinically significant bacteria in the genus are: C. perfringens, C. tetani, C. botulinum and C. difficile. These four Clostridia all produce toxins causing a broad scope of illnesses including; gas-gangrene (myonecrosis) for C. perfringens, tetanus for C. tetani, botulism for C. botulinum and pseudomembranous colitis as well as diarrhea for C. difficile.

Clostridium bacteria are found in large amounts in soil, water, sewage and in the natural flora of humans and animals. These bacteria typically infect skin and soft tissue, cause food poisoning, cause antibiotic-associated diarrhea and colitis. The unfortunately strong ability of Clostridia to cause illness is due to three factors. 1) Their ability to form spores, allowing them to wait out adverse environmental conditions, 2) their rapid growth in anaerobic, nutritionally-rich environments and 3) their production of various toxins; histolytic toxins, enterotoxins as well as neurotoxins.

A small group of Clostridia produce neurotoxins; C. tetani, C. botulinum, C. baratii and C. butyricum. The former two are by far the most important and as such their neurotoxins will be the focus of this paper.

Clostridial Neurotoxins and Their Structure

Clostridial neurotoxins are highly toxic molecules, measured by the LD50 method BoNT is the most lethal toxin in the world with one nanogram per kg killing 50% of the test animals. To put this in perspective the venom of the worlds most venomous snake; the Inland Taipen (Oxyuranus microlepidotus) requires 25 micrograms per kg to achieve the same effect. TeNT is usually considered the second most toxic substance after BoNT.

C. tetani produces a neurotoxin: Tetanus Neurotoxin (TeNT) that causes spastic paralysis by inhibiting inhibitory interneurons in the spinal cord. Other names for TeNT include: Neurospasmin, Tetanospasmin and Spasmogenic toxin.

C. botulinum produce seven distinct botulinum neurotoxins (BoNT) labelled A through G. These toxins inhibit the release of Acetylcholine at neuromuscular junctions in a process that will be explain later in this text.

Both TeNT and BoNT are single chain peptides of 150 000 Da that are cleaved into a heavy chain (B or H chain) of 100 000 Da and a light chain (A or L chain) of 50 000 Da. The peptide chain is cleaved by both by endogenous bacterial and human tissue proteases causing it to become a much more potent toxin than the single chain precursor.

The heavy chain consists of two domains, the carboxylic acid (-COOH) HC domain is responsible for binding to sialic acid and glycopeptides on the surface of cell membranes. The amino (NH_2) HN domain is responsible for moving the light chain zinc endopeptidase out of the endosome.

The light chain consists of a zinc endopeptidase enzyme that cleaves SNARE proteins; proteins involved in the trafficking of cell vesicles and release of neurotransmitters – such as Acetylcholine, GABA and glycine.

Mechanisms of Clostridial Neurotoxins

Due to the similarity between the the mechanism of action of TeNT and the seven BoNTs these will be explained simultaneously with the differences between them being pointed out where relevant. Much of their mechanisms are unclear and this will be pointed out as well.

Clostridial neurotoxins CNTs travel from the site of absorption or production to the presynaptic membrane of cholinergic nerve terminals. TeNT can also bind to sympathetic adrenergic fibers.

CNTs bind to the sialic acid of gangliosides and glycopeptides on the cell surface of peripheral nerves, TeNT and BoNT bind to different sialic acids and glycopeptides than each other. The heavy chain C domain is responsible for this binding and while this step is being researched it seems sialic acid functions as a readily available receptor for which to bind and that the toxin is then moved along the cell surface to also bind with glycoproteins. When both sialic acid and glycoproteins are present the CNT is endocytosed.

The endocytosis of CNTs is mediated by clathrin-coated pits and the proteins RAB5A and RAB7A, these are proteins involved in endosome formation (RAB5A), trafficking and fusion with lysosomes (RAB7A). In the case of peripherally functioning BoNTs the endosome is fused with a lysosome at the peripheral nerve terminal. In the case of the centrally functioning TeNT the endosome is moved to the nerve body in the spinal cord by retrograde endosome transport and from there on to inhibitory interneurons. Exactly how TeNT is moved between nerve soma and interneuron is also unclear but a possible theory noted in Bacterial Protein Toxins by Aktories and Just is via exocytosis of the endosome and release of the toxin into the synapse.

When the endosome has reached its destination it is fused with a lysosome, the acidification of the endosome causes a conformational change of the CNTs and causes the heavy chain N domain to translocate the light chain into the cytoplasm. Again the exact mechanism of how the HN domain translocates the light chain is unclear, but three theories seem plausible. 1)The acidity causes the light chain to unfold, allowing it to move through a pore made by the HN domain. 2)The acidification causes the HN domain to form a channel through the endosome wall resulting in osmotic lysis of the endosome and release of the light chain. 3)The HN domain inserts into a H+ pore and stretches it open allowing the light chain to pass through the pore between membrane lipids and the HN domain. Once it reaches the cytosol the neutral pH causes the light chain to return to its natural configuration.

Once inside the cytoplasm the light chain zinc endopeptidases targets SNARE proteins and degrade them. SNARE proteins mediate vesicle fusion, either with the cell membrane in the case of exocytosis or lysosomes in the case of endolysosome formation. The zinc endopeptidases of TeNT and BoNT are highly specific and target three SNARE proteins at specific nucleotide sequences. TeNT and BoNT B,D,F&G target vesicle-associated membrane proteins VAMP (VAMP1 and 2 are also known as synaptobrevin). TeNT and BoNT B actually target the exact amino acid sequence on VAMP. BoNT A,C&E target SNAP-25 and BoNT C targets both syntaxin and SNAP-25.

The degradation of SNARE proteins affects the endosome and cell membrane interaction at one or more point during docking, priming and fusion. If SNAP-25 is degraded e.g. by BoNT A then docking is inhibited. If VAMP or syntaxin is degraded e.g. by TeNT or BonT C then there is a problem with priming and/ or fusing. Regardless of what point in the docking, priming, fusion sequence is inhibited exocytosis of neurotransmitters will be impaired due to these toxins.

The effects of CNTs depend on whether it is TeNT or BoNT. As mentioned earlier TeNT will affect inhibitory interneurons in the spinal cord, these interneurons release GABA and Glycine and inhibit somatic motor neurons. When this inhibition is lost the motor neurons are overstimulated resulting spastic paralysis (muscle spasms). BoNT acts on peripheral motor neurons and inhibits the release of Acetylcholine at neuromuscular plates resulting in flaccid paralysis.

Conclusion

Not only are CNTs important in the case of their clinical treatment of the diseases that they cause but understanding the mechanisms by which they function allows to use these toxins to gain a better understanding of the molecular biology involved. This understanding allows for the use of CNTs in a clinical manner not only the cosmetic use of BoNT in the form of Botox but also understanding for example the retrograde transport of vesicles by attaching molecules to TeNT. SNARE protein function can also be assessed by using CNTs selective for them, the function of inhibitory interneurons also becomes more clear by the administration of TeNTs and BoNTs are being used to treat muscle spasms. As this was written CNTs were also being researched various other uses and the contribution of these toxins to science is likely to increase with time.

Sources:

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