جامعة الانبار

كليه العلوم

قسم علوم الحياة

Bacterial toxins

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Lecture 8

Tetanus toxin

سموم التيتانس

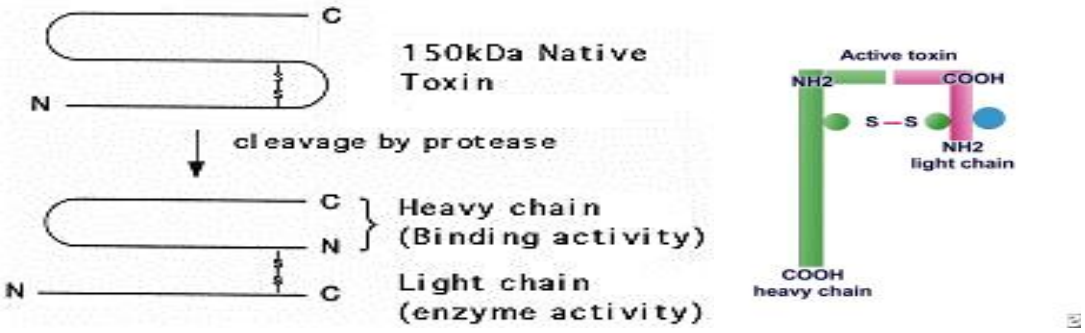
Tetanus toxin Lec8

Tetanus toxin is the neurotoxin produced by the vegetative cell of *Clostridium tetani* in anaerobic conditions, causing tetanus. It is sometimes called spasmogenic toxin, tetanospasmin or abbreviated to TeTx or TeNT.*C. tetani* also produces the exotoxin tetanolysin, the effects of which are as yet unclear.The genes encoding these toxin are located on separate plasmids within the bacterium.

Tetanus toxin spreads through tissue spaces into the lymphatic and vascular systems. It enters the nervous system at the neuromuscular junctions and migrates through nerve trunks and into the central nervous system (CNS) by retrograde axonal transport by using dyneins.

Structure

The protein tetanus toxin has a molecular weight of 150kDa. It is made up of two parts: a 100kDa heavy or B-chain and a 50kDa light or A-chain. The chains are connected by a disulfide bond.



- The B-chain binds to disialogangliosides (GD2 and GD1b) on the neuronal membrane.
- The A-chain, a zinc endopeptidase, attacks the vesicle-associated membrane protein (VAMP).

ToxinAction

Tetanospasmin initially binds to peripheral nerve terminals. It is transported within the axon and across synaptic junctions until it reaches the central nervous system. There it becomes rapidly fixed to gangliosides at the presynaptic inhibitory motor nerve endings, and is taken up into the axon by endocytosis. The effect of the toxin is to block the release of inhibitory neurotransmitters (glycine and gamma-amino butyric acid) across the synaptic cleft, which is required to inhibit nervous impulse. If nervous impulses cannot be checked by normal inhibitory mechanisms, it produces the generalized muscular spasms characteristic of tetanus. Tetanospasmin appears to act by selective cleavage of a protein component of synaptic vesicles, synaptobrevin II, and this prevents the release of neurotransmitters by the cells.

The receptor to which tetanospasmin binds has been reported as ganglioside GT and/or GD1b, but its exact identity is still in question. Binding appears to depend on the number and position of sialic acid residues on the ganglioside. Isolated B fragments, but not A fragments, will bind to the ganglioside. The A fragment has toxic (enzymatic) activity after the B fragment secures its entry. Binding appears to be an irreversible event so that recovery depends on sprouting a new axon terminal.

The action of the A-chain stops the affected neurons from releasing the inhibitory neurotransmitters GABA (gamma-aminobutyric acid) and glycine by degrading the protein synaptobrevin. The consequence of this is dangerous overactivity in the muscles from the smallest stimulus—the failure of inhibition of motor reflexes by sensory stimulation. This causes generalized contractions of the agonist and antagonist musculature, termed a tetanic spasm.

Clinical significance

Tetanic spasms can occur in a distinctive form called *opisthotonos* and be sufficiently severe to fracture long bones. The shorter nerves are the first to be inhibited, which leads to the characteristic early symptoms in the face and jaw, *risus sardonicus* and lockjaw.

The toxin bind to the neurons is irreversible and nerve function can only be returned by the growth of new terminals and synapses.

Immunity

Unlike other toxigenic diseases, such as diphtheria, recovery from the natural disease usually does not confer immunity, since even a lethal dose of tetanospasmin is insufficient to provoke an immune response.

Prophylactic immunization is accomplished with tetanus toxoid, as part of the DPT (DTaP) vaccine or the DT (TD) vaccine. Three injections are given in the first year of life, and a booster is given about a year later, and again on the entrance into elementary school.

Whenever a previously-immunized individual sustains a potentially dangerous wound, a booster of toxoid should be injected. Wherever employed, intensive programs of immunization with toxoid have led to a striking reduction in the incidence of the disease.

The Botulinum Toxins

Neurotoxin production is the unifying feature of the species *C. botulinum*. Seven types of toxins have been identified and allocated a letter (A-G). Most strains produce one type of neurotoxin but strains producing multiple toxins has been described. Different serotypes have distinct protein structures, modes of action and potencies. The most potent serotype is type A and this is the more commonly used toxin in medicine. Serotype B is also available as a medicine.

Botulin toxin produced by *Clostridium botulinum* is often believed to be a potential bioweapon as it is so potent that it takes about 75 nanograms to kill a person (LD50 of 1ng/kg, assuming an average person weighs ~75kg); 500 grams of it would be enough to kill half of the entire human population.

Botulinum toxin is secreted under anaerobic conditions (without oxygen) by a bacterium commonly found in soil called *Clostridium botulinum*. In nature the toxin is found in association (complexed) with non-toxic proteins which include haemagglutinin proteins.

Botulinum toxin blocks transmission of messages from nerves to muscles and therefore weakens muscles temporarily.Accidental ingestion of large quantities of botulinum toxin e.g. from improperly canned foods can lead to an acute paralytic illness called botulism.

Structure of botulinum toxin

Botulinum toxins are proteins that have similar molecular structures and weights of around 140-170 kDA. The toxins are produced as a single polypeptide chain, weighing approximately 150 kDA, and are only weakly toxic. The chain is activated by a process of proteolytic cleavage, to form a di-chain molecule (comprised of a heavy chain and a light chain), linked by a disulphide bond.

The light chain (~50 kD - amino acids 1-448) acts as a zinc (Zn^{2+}) endopeptidase similar to tetanus toxin with proteolytic activity located at the N-terminal end (see image below). The heavy chain (~100 kD - amino acids 449-1280) provides cholinergic specificity and is responsible for binding the toxin to presynaptic receptors; it also promotes light-chain translocation across the endosomal membrane.

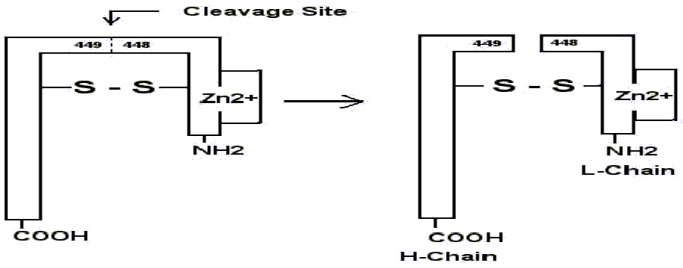


Fig: - Structure of botulinum toxin B ,Proteolytic activity is located at the N-terminal end of the light chain of botulinum toxin type A.

Mode of action of botulinum toxin

The botulinum toxins are very similar in structure and function to the tetanus toxin, but differ dramatically in their clinical effects because they target different cells in the nervous system. Botulinum neurotoxins predominantly affect the peripheral nervous system reflecting a preference of the toxin for stimulatory motor neurons at a neuromuscular junction. The primary symptom is weakness or flaccid paralysis.

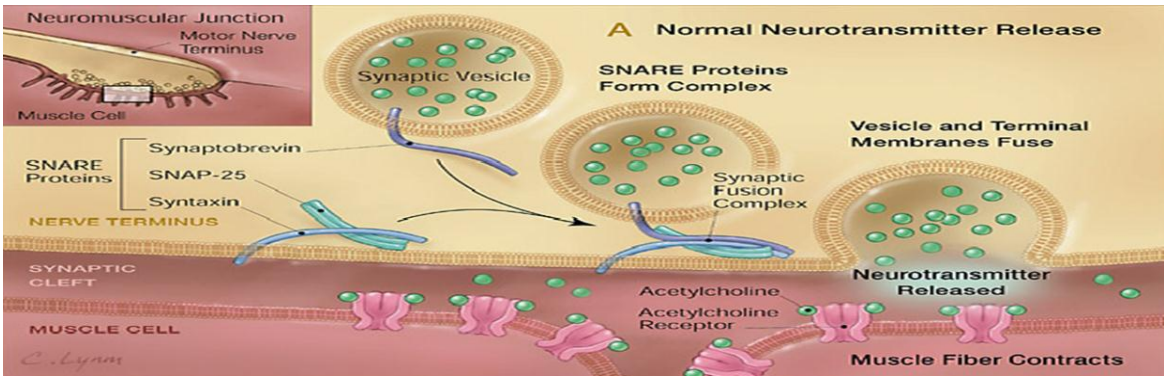
Tetanus toxin can affect the same system, but the tetanospasmin shows a tropism for inhibitory motor neurons of the central nervous system, and its effects are primarily rigidity and spastic paralysis**.**

Normal neuromuscular transmission

Acetylcholine is the primary neurotransmitter operating at the neuromuscular junction. Vesicles containing acetylcholine are stored in the presynaptic terminal of the nerve. Before releasing acetylcholine, the vesicles need to be transported to, and fuse with, the nerve terminal membrane. The transport of synaptic vesicles to the terminal membrane, and the eventual release of acetylcholine, is controlled by a group of proteins called the SNARE complex - (the Soluble N-ethylmaleimide-sensitive factor attachment protein receptor complex). The SNARE complex consists of a number of proteins, but the three main ones are:

- SNAP-25 (synaptosomal-associated protein of 25kDa)
- Synaptobrevin (also known as vesicle-associated membrane protein or VAMP)
- Syntaxin

Following fusion of the vesicle with the synaptic membrane, acetylcholine is released into the synaptic gap, diffuses across the gap, and binds to nicotinic cholinergic receptors on the postsynaptic membrane, triggering muscle contraction.



Binding and internalization of botulinum toxin

When botulinum toxin is injected into the muscle it has significant affinity for peripheral cholinergic nerve endings, where it binds rapidly, specifically and irreversibly to receptors on the presynaptic membrane - the heavy chain facilitates this binding and internalisation of the toxin. The bound toxin is taken up into the nerve terminal by endocytosis. The resulting endosome containing toxin migrates into the cytosol and at this point it splits into a heavy chain and an active light chain.

Inhibition of acetylcholine release

The active light chain of botulinum toxin has a specific affinity to cleave certain proteins involved in the mechanism of acetylcholine exocytosis. Botulinum toxin type A cleaves SNAP-25 so that:

- Acetylcholine vesicles cannot fuse with the presynaptic membrane
- Acetylcholine is not released
- Neuromuscular transmission is blocked
- Muscle weakness and paralysis occurs

Reference

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