Botulism and Membrane Fusion

What is the biochemical mechanism whereby botulism toxin causes an often-fatal muscle paralysis? *Botulism* is a form of muscle paralysis caused by a protein toxin produced by the anaerobic bacterium *Clostridium botulinum*. The toxin usually enters the body either by the consumption of improperly sterilized canned foods or by the contamination of a wound by the bacterium. Muscle paralysis begins in facial muscles, causing symptoms such as loss of facial expression and difficulty with swallowing and speech. Death may be caused by respiratory failure, the result of paralysis of the intercostal muscles that move the chest wall. The botulinum toxin prevents the release of the neurotransmitter acetylcholine (ACH) from the presynaptic axon of motor neurons. It does so by interfering with the fusion of ACH-containing synaptic vesicles with the nerve cell membrane. After a brief overview of the membrane fusion process, botulinum toxin mechanism is described.

Membrane Fusion

Membrane reorganization is a constant operational feature of eukaryotic cells. In the endomembrane system (p. 44), biosynthetic and secretory processes involve the movement of biomolecules through a series of transfers from one membrane–bound compartment to another. Molecules such as digestive enzymes and hormones, for example, are processed through the ER and Golgi complex and are eventually secreted from the cell by exocytosis (Figure 2.15). At each transfer step, these substances are passed from a donor compartment to a target compartment in a process that involves the fusion of membranes. Membranous vesicles pinch off from the donor compartment, and after transport by the cytoskeleton to their destination, the vesicles deliver their cargo by fusing with the membrane of a target compartment.

Membrane fusion, the merging of two lipid bilayers, is a precise and highly regulated process that is made possible by a specialized set of proteins that function as a fusion machine. The **SNAREs** (*s*oluble *N*-ethylmaleimide sensitive factor *a*ttachment protein *r*eceptors), the most essential components of the fusion machinery, are a large class of small transmembrane proteins (18–42 kDa), each consisting of a membrane bound C-terminal domain and a helical domain that extends into the cytoplasm. (*N*-Ethylmaleimide is a reagent used to elucidate the functional properties of the fusion proteins.) There are two categories of SNAREs: v-SNAREs (vesicle-specific proteins) and t-SNAREs (target membrane proteins). The principal features of the fusion mechanism are as follows.

As a vesicle (the donor membrane) approaches the target membrane, the helices of the v-SNARE and the t-SNARE interact, forming four helix bundles (the core complex) from relatively unstructured helices (**Figure 1**). The "zippering" of each set of coiled coil structures creates a torsional force that draws the opposing membranes into intimate contact in a process that expels water molecules (i.e.,

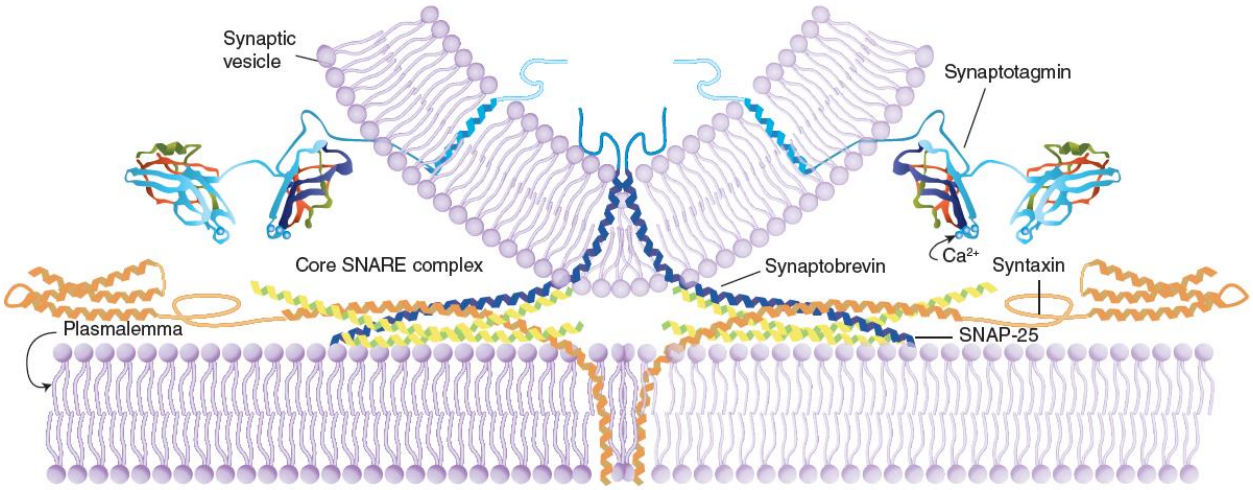

FIGURE 1

Membrane Fusion

The fusion of neurotransmitter vesicles with the presynaptic membrane of neurons begins with the formation of the core SNARE complex from synaptobrevin (a v-SNARE), syntaxin (a t-SNARE), and two helices of SNAP-25, a peripheral membrane protein component of the t-SNARE complex. Synaptotagmin is a Ca^{2+} sensor that triggers the late stages of membrane fusion when local Ca^{2+} levels are high.

hydrostatic pressure is overcome). Once assembled, the fusion machinery is activated when synaptotagmin, a Ca^{2+}-sensor protein in the vesicle membrane, undergoes a conformational change that is triggered by a localized rise in calcium levels. The now-active fusion machinery promotes the rearrangement of the two lipid bilayers to form the fused membrane. After fusion is complete, the SNARE complexes are disassembled by *N*-ethylmaleimide sensitive factor (NSF). NSF, an ATPase, contains a clamp-like module that in combination with α-SNAP (*s*oluble *N*SF *a*ttachment *p*rotein) exerts the mechanical force necessary to pry the stable SNARE complex apart so that their components can be recycled.

The Botulinum Toxin Mechanism

Botulinum toxin consists of a heavy chain (100 kDa) linked by a disulfide bridge to a light chain (50 kDa). All seven toxin types (A, B, C, D, E, F, and G) inhibit the release of ACH from motor neurons. The toxin enters the cell via endocytosis triggered by the binding of the heavy chain to a plasma membrane receptor. The light chain exits the endocytotic vesicles and migrates to the presynaptic membrane, where it cleaves a SNARE protein, thereby disabling the fusion machinery. Each toxin type disables a specific membrane fusion protein. For example, toxins A and B cleave SNAP-25 (a t-SNARE) and synaptobrevin (a v-SNARE), respectively.

SUMMARY: Botulinum toxin causes muscle paralysis by preventing the membrane fusion event that releases the neurotransmitter ACH into the neuromuscular junction.