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Molecular Mechanism of the Action of *Clostridium botulinum* Type B Neurotoxin

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Clostridium botulinum neurotoxin (BoNT) has been classified into seven immunological types from A through G. BoNT exerts its toxin action by inhibition of neurotransmitter release, which results in neuromuscular paralysis. BoNT is produced as a single polypeptide chain with a molecular mass of 150 kDa. The neurotoxin in a nicked form, is made up of two chains, the heavy (100 kDa) and light (50 kDa) chains, which are covalently linked by a disulfide bond. It has been proposed that BoNT action involves the following steps, binding to receptors on the presynaptic membrane, subsequent internalization, and translocation into cytosol where the light chain acts by inhibiting neurotransmitter release. Based on the recent studies, it has been proposed that the light chain of all types exhibit zinc-dependent protease activities toward one of three neural protein, VAMP (vesicle-associated membrane protein)/synaptobrevin, syntaxin/HPC-1, and SNAP-25 (synaptosomal-associated protein of 25 kDa). The findings provided the direct evidence that VAMP, syntaxin, and SNAP-25 have a crucial role in synaptic vesicle exocytosis. Since these proteins form a stable core complex during synaptic vesicle docking, their cleavage by the light chain causes a failure of the precise assembly and induces instability of the complex, suggesting that the formation of the synaptic core complex is essential for synaptic vesicle exocytosis.

In comparison with the intracellular events, it is still obscure how BoNT recognizes specific component(s) on the surface of presynaptic membrane, although the BoNT binding to plasma membrane is an essential first step for the development of paralysis. One reason is probably the complexity of the receptor. Competition experiments with different types of BoNTs showed that they bind to type specific components. We have purified type B BoNT (BoNT/B) binding proteins from rat brain and identified them as synaptotagmins I and II. However, BoNT/B did not bind to synaptotagmin alone, but was only observed in the presence of ganglioside GT1b or GD1a, suggesting that synaptotagmin form the toxin binding site by associating with the specific gangliosides. Recombinant deletion mutants of synaptotagmin II allowed us to demonstrate that the N-terminal domain retains BoNT/B binding activity. There were several reports that GT1b binds to BoNTs and causes loss of toxicity without toxin type specificity. In order to clarify its role in constituting as a component of receptor, we examined the inhibitory effect of a monoclonal antibody against GT1b on BoNT binding to receptor and toxic action to rat superior cervical ganglion neurons. The antibody antagonized the action the actions of both BoNT/A and BoNT/B. These data indicate that GT1b functions as a common and complementary components for BoNT receptors. Protein components like synaptotagmin appear to define type specificity of the

BoNT receptor.

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