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Seeing a Neurotoxin's Deadly Grip

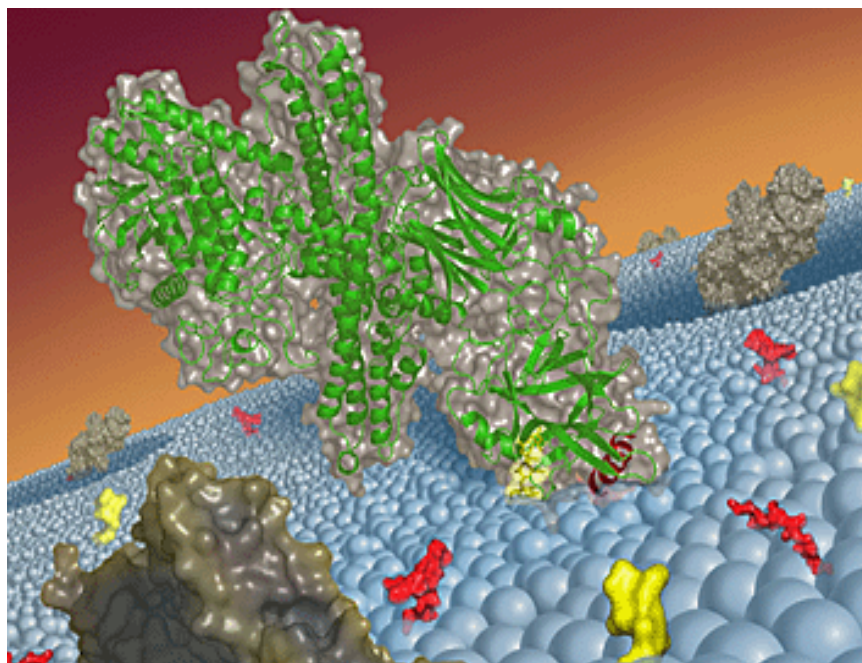


Image Title: Botulinum neurotoxin hijacks synaptic vesicle recycling at neuromuscular junctions. The toxin first docks to the active zone (blue) by binding to two membrane-anchored receptors, synaptotagmin (red) and ganglioside (yellow). The toxin-receptor complexes are then internalized by endocytosis. - Courtesy of Axel Brunger, HHMI at Stanford University

Two Howard Hughes Medical Institute research teams working independently have discovered new information about how the botulinum neurotoxin shuts down neurons with deadly efficiency. By providing detailed views of the toxin plugged into its neuronal receptor, the new studies could aid efforts to engineer specialized versions of the powerful neurotoxin that is used to treat a wide array of medical problems.

The two groups were led by HHMI investigators Axel Brunger at Stanford University and Edwin Chapman at the University of Wisconsin at Madison. They published their findings December 13, 2006, in advance online publications in the journal *Nature*.

“Botulinum neurotoxins are powerful tools for biologists and find widespread use as therapeutics for the treatment of certain nervous-system diseases,” wrote Giampietro Schiavo of the London Research Institute in an accompanying *News & Views* commentary in *Nature*. “For these reasons, the papers reported here are of tremendous value.”

"These toxins could prove the ideal basis for drugs, because they work at extremely low concentration."

- Axel T. Brunger

Botulinum neurotoxins are among the most deadly natural toxins in the world. They act by first attaching themselves to receptors on the surface of neurons. The toxins then insinuate an enzyme into the neuron that degrades key proteins required for neurons to communicate with one another. The toxins principally affect muscle-controlling motor neurons activated by the neurotransmitter acetylcholine. They kill by paralyzing the respiratory muscles. There are seven structurally and functionally related botulinum neurotoxins (BoNTs), called serotypes A through G, with each acting in a slightly different manner. In 2004, Brunger's group published an article in *Nature* detailing how the toxins that cause botulism and tetanus can recognize and attack particular nerve cell proteins at the neuromuscular junction.

Researchers knew that the toxins simultaneously bind to two distinct neuronal receptors - one a protein and one a sugar-containing lipid called ganglioside - but the details of that binding had not been established prior to these studies.

Both research groups began by crystallizing the BoNT/B serotype toxin in complex with its protein receptor, called synaptotagmin II. Working with Chapman's group, co-authors Raymond Stevens, Qing Chai and Joseph Arndt of the Scripps Research Institute crystallized full length BoNT/B in complex with the “recognition domain” of synaptotagmin II, to which the toxin attaches. Simultaneously, working in Brunger's group, lead author Rongsheng Jin, an HHMI postdoctoral fellow, in collaboration with Thomas Binz and Andreas Rummel of the Medizinische Hochschule Hannover in Germany crystallized the receptor-binding domain of BoNT/B in complex with the recognition domain of synaptotagmin II, achieving a significantly higher resolution of the complex.

Each research group determined the structure of the toxin-domain complex using x-ray crystallography. In x-ray crystallography, protein crystals are bombarded with intense beams of x-rays. As the x-rays pass through and bounce off of atoms in the crystal, they leave a diffraction pattern, which can then be analyzed to determine the three-dimensional shape of the protein.

Both groups discovered that the toxin holds its receptor in an intimate molecular embrace. The toxin induces a helix in the synaptotagmin protein that fits precisely into a groove in the toxin molecule. Both teams showed that they could disrupt this binding by introducing mutations that would subtly alter the shape of the synaptotagmin receptor.

Brunger and his colleagues found that altering the toxin at the binding site by single amino acid changes (obtained from the high resolution crystal structure) drastically reduced its toxicity. Specifically, when they incubated the altered neurotoxin with mouse diaphragm, it produced far less muscular paralysis than the natural toxin.

“This tells us that it is possible to design a small-molecule inhibitor that could powerfully disrupt the interaction between the toxin and the receptor,” said Brunger. “Such inhibitors would act as powerful, specific anti-toxins, with fewer side effects than current drugs” Also, he said, detailed knowledge of toxin-receptor binding could help in designing botulism vaccines. Such vaccines could consist of fragments of protein corresponding to the toxin's binding region, which could be used to trigger antibody production against the toxin that would block its action.

Both research groups also explored the role of the ganglioside binding site in the toxin's double-receptor binding. Brunger and his colleagues found that the protein and ganglioside binding sites are quite distinct, although they are both necessary for the toxin to attach to the neuron and enter it.

Both groups found that docking between the toxin and the double sites is more extensive than previously believed. “It's like nestling a cube into a corner,” Chapman said. “The surfaces mesh perfectly, so that the toxin is juxtaposed very near the membrane in preparation for translocation into the cell. Practically speaking, this knowledge of how the toxin recognizes the double receptor could lead to a rational basis for designing small-molecule inhibitors to this recognition step,” he said.

Brunger and Chapman emphasized that discoveries about the toxin-receptor structure could lead to significant advances in engineering the toxin for clinical application. Injection of the toxin is already widely used to erase wrinkles and frown lines. However, the toxin is also used to treat migraine headaches, involuntary contraction of the eye muscles, overactive bladder syndrome, excessive sweating and spastic disorders associated with injury or disease. The researchers see the potential for even more widespread use.

“These toxins could prove the ideal basis for drugs, because they work at extremely low concentration,” said Brunger. “The therapeutic doses are less than a nanogram per kilogram of body weight - compared to milligram-per-kilogram concentrations needed for most pharmaceutical drugs.”

“With knowledge of the toxin-receptor interaction, you can begin to design toxin-receptor pairs to change the specificity of what cell gets recognized,” said Chapman. “You can also change the specificity of the protein the toxin cleaves inside the cell.” Thus, he said, engineered toxins could provide targeted treatments for a wide array of medical disorders.”