

# Toward a Kinder, Gentler Toxin

*New research findings may ultimately broaden the beneficial uses of botulinum toxin and help protect people from its threat as an instrument of terrorism.*

THE POISON PRODUCED BY *CLOSTRIDIUM BOTULINUM* HAS UNDERGONE A reputation makeover—from feared to favored. In the 1950s, food-borne botulism killed one in four of its victims; half a century later, more than 3 million Americans paid for commercial Botox injections to smooth their wrinkles and frown lines. ¶ Botulinum could have far greater impacts at both ends of this spectrum.



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EDWIN CHAPMAN

Its potential as a vehicle for innovative medical therapies, or as a devastating weapon of mass destruction, imparts a special urgency to the work of two HHMI scientists.

HHMI investigator Edwin R. Chapman, a physiology professor at the University of Wisconsin–Madison, and biophysicist Axel T. Brunger, an HHMI investigator at Stanford University, are broadly interested in neurotransmission—the passage of signals from one nerve cell to another. Both have been looking specifically at how botulinum neurotoxins (BoNTs) impair the release of chemical neurotransmitters at junctions between nerves and muscles, resulting in paralysis that ranges from therapeutic to lethal.

“These are among the most potent toxins on earth,” says Chapman. “Once we’ve figured out how they do what they have

evolved to do, we can begin working on getting them to do what we *want* them to do.” Applications might include antitoxins that combat botulism poisoning, vaccines that prevent it, and a range of disease therapies derived from a better understanding of botulinum’s efficiency in targeting and shutting down neurons.

The latest findings by Chapman and Brunger build on earlier research indicating that BoNTs attach to their target neurons in a high-affinity bind involving proteins and gangliosides (sugar-containing lipids). In 2003, Chapman’s group singled out a protein called synaptotagmin II as a cell entry mediator for BoNT/B, one of the seven types of BoNT. Brunger, in 2004, showed how BoNT/A recognizes and ensnares its target protein on the nerve cell; in 2006, Chapman’s group published its discovery of the protein receptor for BoNT/A.

This past December, Brunger and Chapman both presented crystal structures of BoNT/B binding in papers published simultaneously in *Nature*. The groups

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## The Good, the Bad, and the Ugly

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**AS THE FIRST BIOLOGICAL TOXIN** approved for the treatment of human disease, botulinum has already addressed an impressive array of conditions. It is licensed as a therapy for involuntary neck-muscle spasms and eyelid contractions, crossed eyes, and abnormally heavy perspiration. Experimental uses—some with promising results—include treatment of migraine headaches, chronic low back pain, stroke, and cerebral palsy. National Public Radio talk show host Diane Rehm has spoken extensively about the botulinum treatment she receives for spasmodic dysphonia, a larynx-muscle disorder that nearly ended her career. >> **MEANWHILE, HISTORY SUGGESTS** that the potential use of botulinum toxin as a biological weapon should be a continuing concern. *The Journal of the American Medical Association* published a report months before the 9/11 attacks that summarized the threat in a short history—from Japan’s testing of botulinum toxins on prisoners in Manchuria in the 1930s to Iraq’s 1991 revelation to United Nations inspectors that it had created enough botulinum to kill the world’s population three times over. —T.T.

used different approaches to crystallize the complex between BoNT/B and synaptotagmin II. Brunger's group collaborated with researchers from Germany's Medizinische Hochschule, Hannover (Hannover Medical School); Chapman worked with Ray Stevens of The Scripps Research Institute.

With resulting crystal structures that were very similar, both teams found that synaptotagmin II forms a short helix that binds to a water-repellent groove within BoNT/B. And both found that just a slight change, or mutation, of the synaptotagmin receptor disrupts the binding.

Brunger's team altered the protein by single amino acids, which they selected based on their crystal structure, and then

tested the effect on the nerve that causes the diaphragm to contract in mice. "We found that just single mutations can lower the toxicity of BoNT/B a thousand-fold," he says. Their work suggests that a small-molecule inhibitor could be designed to interfere with the binding of botulinum toxin and avert lethal paralysis of the diaphragm.

Both researchers are energized by the broader prospects of the research. Chapman's inspiration is to see the creation of a mutant toxin that would not affect regular cells but would bind to an engineered mutant receptor for targeted use in the body. "By persuading the toxins to act only on cells that have been sensitized to them, we hope to further harness their usefulness as medicines and as research tools," he says.

Meanwhile, given the threat posed by botulinum as a biological weapon, U.S. military and security officials are very interested in the development of inhibitors or vaccines. The current state of the art is limited to two problematic options: an equine antitoxin that



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AXEL BRUNGER

carries a significant risk of adverse reaction in humans, and an investigational detoxified botulinum toxin that prevents botulinum poisoning but also renders Botox and other botulinum treatments ineffective.

■ -TRACIE THOMPSON



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