

MARCH 16, 2006

Botulism Toxin's Insidious Route into Nerve Cells

Botulinum neurotoxin A can be either the greatest wrinkle remover or one of the world's most potent biological weapons. To perform either job, however, the toxin must first find a way to enter cells.

But understanding how the toxin -- one of seven neurotoxins produced by the bacterium *Clostridium botulinum* -- enters nerve cells has proved elusive for scientists. Despite a decade-long search for the receptor by labs around the world, researchers had come up empty handed.

Now, a research team led by Howard Hughes Medical Institute (HHMI) researcher Edwin R. Chapman reports that it has identified the cellular receptor for botulinum neurotoxin A. The group's work was published in the March 16, 2006, edition of *ScienceExpress*, which provides electronic publication of selected *Science* papers in advance of print. The finding offers important new insights that suggest how the toxin shuts down nerve cells with deadly efficiency.

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In the clinic, the toxin, which is also known as botox, is used to treat forehead wrinkles, migraine headaches, urinary retention, eye muscle disorders, and excessive sweating. The same toxin also has more nefarious uses, and is considered a potential bioterror threat because it can kill people by paralyzing motor nerves in diaphragm muscles, causing breathing to stop. Lack of knowledge about the identity of the cell surface receptor that botulism toxin A uses to invade nerve cells has hindered the development of new antidotes to the toxin.

"People thought that since these were the most potent toxins known to humans, it would be easy to find the receptors," said Chapman, whose HHMI

laboratory is at the University of Wisconsin-Madison. However, only a handful of proteins had been identified that appeared to interact with the toxin. But none of these proteins turned out to be the receptor, he said.

According to Chapman, researchers had long known how botulinum neurotoxin A attacks the nerve cell's internal molecular machinery. But the identity of the neuronal surface protein that the toxin recognized and used to gain entry into the cell was unknown.

“We decided to study the entry route used by these toxins first,” said Chapman. Using cultured neurons and mouse diaphragms as model systems, postdoctoral fellow Min Dong and Felix Yeh in Chapman's laboratory, revealed that the neurotoxin enters neurons when empty synaptic vesicles are being recycled from the cell surface to the cell's interior. Synaptic vesicles are sac-like cargo carriers in neurons that haul neurotransmitters from the cell's interior to the synapses, which are the junctions between neurons. At the synapse, neurotransmitters are released, triggering nerve impulse in neighboring neurons.

“Our uptake experiments with all the toxins showing that many of them are taken up through synaptic vesicles made our life simple, because almost all synaptic vesicle proteins had already been identified by our colleagues. Furthermore, there are only a handful of synaptic vesicle proteins that contain domains that are exposed on the cell surface,” said Chapman.

Thus, when Dong and Yeh screened the major vesicle proteins for binding to the neurotoxin, they found a high level of specific binding to one called SV2. Furthermore, the researchers found they could block the toxin's action in neurons by adding the piece of the SV2 protein that they had discovered was the SV2 protein's binding site to the toxin.

The researchers then proceeded to study the interaction between the toxin and SV2 in cell cultures, tissues and in whole mice. Co-author Roger Janz of the University of Texas-Houston Medical School supplied the Wisconsin researchers with knockout mice that lacked certain versions of SV2. The Wisconsin group found that the neurons that lack SV2 do not take up botox, but they do take up the toxin when SV2 is expressed. These findings demonstrated that SV2 is the functional receptor for Botox, Chapman said.

Other key mouse experiments were done in the laboratory of co-authors Eric Johnson and William Tepp in the Food Research Institute at the University of Wisconsin. They found that mice engineered to lack versions of the SV2 protein showed significantly longer survival times than did normal mice when exposed to the toxin.

The identification of SV2 as the neurotoxin A receptor raises the possibility of designing protective drugs that would interfere with the toxin's action, said Chapman. He said his laboratory will aid such efforts by concentrating on

developing a more detailed understanding of the molecular interaction between the toxin and its receptor.

Chapman said that this finding and others' studies on the botulinum neurotoxins have revealed why they are models of lethal efficiency. "The cool thing is that the neurotoxin receptor is on actively recycling synaptic vesicles, so the toxin targets only active neurons and shuts them down," he said. "There is no wasted toxin, because once a nerve terminal is shut down, it doesn't take up any more toxin. That leaves more toxin around to enter nerve terminals that have yet to be inhibited. That's pretty clever."