

- beauty and poison -

Oliver G Weingart, 2009

1895
Emile van Ermengem first isolates *Clostridium botulinum* and correlates its toxin with botulism.

1949/50
Dr. Burgen finds out that BoNT blocks neuromuscular transmission.
Dr. Brooks discovers that BoNT inhibits Acetylcholine release.

1989
BOTOX® (BoNT/A) approved by FDA for treatment of strabismus, blepharospasm, hemifacial spasm in children.

1817
Justinus Kerner first describes the bacterium *Bacillus botulus** being responsible for sausage poisoning cases.
*botulus is latin and means sausage

1946
Dr. Edward Schantz succeeds in purifying BoNT/A in crystal-line form.

1973/80
Dr. Alan Scott uses BoNT in monkey experiments, later in humans to treat strabismus.

Toxins

BACTERIA	PLANT	FUNGI	ANIMAL
Clostridial neurotoxins	Solanin	Muskarin	Tetrodotoxin (blowfish, frogs)
<i>B. cereus</i>	Atropin	Gyromitrin	Snake venom
<i>S. aureus</i>	Saxitoxin (algae/shellfish)	Amanitin	Conotoxins (cone snails)
Salmonella	Ricin, Abrin	Orellanion	Batrachotoxin (frogs)
<i>E. coli</i> (EHEC)	+ many others	Aflatoxine	+ others
+ many others			

Structure

- purified toxin build by a **heavy chain (HC)** with 100kD and a **light chain (LC)** with app. 50kD

Purified botulinum neurotoxin (left) and complexed botulinum neurotoxin (right) showing the heavy chain (HC) and light chain (LC) with Zn²⁺ ions and disulfide bridges.

- Botulinum neurotoxin is secreted in to the medium and associates with non-toxic clostridial proteins
- ⇒ large **toxin complex** is formed (up to 900kD in size)

Definition of Toxins

(greek: *toxikon* = arrow poison)

- produced by plant, animals or bacteria
- often proteins (high molecular weight)
- antigenic properties
- highly toxic
- not contagious (unlike bacterial/viral infections)

Genetic Diversity

- There are 7 distinct serotypes (named A-G) and further subtypes of botulinum neurotoxin which are produced by different strains of *Cl. botulinum*
- They can differ in genetic and amino acid sequence, protein structure, and to a certain degree in their function

Bacteria

- Clostridium botulinum* strains
 - C. botulinum*
 - C. baratii*
 - C. argentinense*

Toxin / Complex Serotypes

- BoNT/A, BoNT/B, BoNT/C, BoNT/D, BoNT/E, BoNT/F, BoNT/G

Subtypes

- A1, A2, A3, A4
- B1, B2, B3, B4
- C1, C/D
- D, D/C
- E1, E2, E3
- E: It butyr⁺, E Ch butyr⁺
- F baratii, Proteolytic F, Bivalent F, Nonproteolytic F

Smith et al., 2005 / H8 et al., 2006

Clostridium botulinum

- anaerobic and sporeforming bacterium
- ubiquitously** found in soil and marine sediments
- toxin produced by *Cl. botulinum*, *Cl. baratii* and *Cl. butyricum*
- every toxin producing strain has a non-toxic counterstrain

Botulism

- If botulinum neurotoxin is ingested it goes from the intestinal tract to the blood system
- Via the blood system botulinum neurotoxin reaches cholinergic nerve cells at neuromuscular junctions
- Heavy chain of botulinum neurotoxin binds two receptors and is internalized

Recognition of membrane receptors and internalization of BoNT takes place.

- SNARE proteins are cleaved by Light chain so that acetylcholin secretion is blocked
- following muscle becomes paralysed leading to the disease called **botulism**
- Botulism starts in sensitive muscles like eye and tongue muscle, then reaches limb muscles and terminally can lead to death by pulmonic arrest

Motorisches Neuron: Acetylcholin (ACh) vesicles, SNAP-25, Syntaxin, SNARE-Proteine, Membranfusion, ACh-Rezeptor, Muskelzucker, Myosin, Depolarisation, Kontraktion, Lähmung.

Detection

- Botulinum neurotoxin and its complex can be detected via immunological techniques like ELISA or more sophisticated systems using magnetic bead separation and/or bead recognitions systems like Bio-Plex 200 with a detection limit of a few picogramm (1pg = 10⁻¹²g)
- Botulinum neurotoxins can also be detected with MALDI-TOF Masspectrometry via its endoproteinase activity
- However, the Golden Standard for botulinum neurotoxin detection still is the Mouse lethality assay with a sensitivity of ~5pg

Botulism and Bioterror

- Of the 7 botulinum neurotoxin serotypes mostly types A, B, E, and F are toxic to humans and lead to botulism
- Although there is no certain data on lethal doses of the toxin it is assumed, that oral lethal dose is app. 0.7-1µg per person. The intraperitoneal dose is estimated to be app. 70ng per person, similar to the lethal dose by inhalational intoxication.
- Due to its extremely low lethal dose, botulinum neurotoxin is the most toxic substance known to man and therefore poses the risk of being used as a possible weapon for bioterrorism
- Fortunately, botulinum neurotoxin is readily inactivated by normal fresh water treatment (UV, ozone), by 30 min at sunlight and by few minutes cooking and proper canning
- Recent results indicate, that common pasteurisation is also sufficient to destroy botulinum neurotoxin to a great extent