Bahman Jabbari

Botulinum Toxin Treatment

What Everyone Should Know

Second Edition

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Preface

Botulinum toxin therapy is now a major mode of treatment for a variety of medical ailments. The notion that a potent toxin can be used in a safe way to treat so many medical disorders was unimaginable up to 50 years ago. It was through tireless efforts of basic scientists and clinical researchers that the use of this unusual, but effective mode of therapy achieved acceptance in the medical community. Botulinum toxin therapy is now used worldwide in the management of millions of patients. This book describes different approved indications or potential indications of botulinum toxin therapy in a language that would be comprehensible by non-medical individuals. Wherever possible, medical terms are explained in a language understandable to the public.

Since the first edition of this book 5 years ago, a large literature has developed describing the long-term efficacy and safety of toxin therapy in the approved indications such as migraine, bladder disorders and spasticity as well as emergence of more data on potential important indications for this mode of treatment such as efficacy in depression and in certain heart ailments (atrial fibrillation). FDA has approved several toxins (Botox, Dysport and Xeomin) for treatment of spasticity in children with cerebral palsy and other spasticity producing conditions. FDA also approved two new toxins for use in the United States: Jeuveau and Daxxify; Jeuveau has been approved only for cosmetic use, whereas Daxxify was approved for cosmetic use as well as treatment of cervical dystonia—a common movement disorder affecting the neck, often associated with disabling neck pain.

The current edition of the book includes two new chapters, one on the use of botulinum toxin therapy in dentistry and the other on the growing use of toxin therapy in veterinary medicine for canine and equine pains. All chapters of the book are written by Bahman Jabbari M.D. with the exception of Chap. 13 on the cosmetic and aesthetic indications that is provided by Drs Marie Noland and Marissa Dennis. In this edition, the information on safety of botulinum toxin therapy has been considerably expanded with inclusion of new data in Chap. 17. Chapter 19 of this book also provides substantially more information regarding potential indications of botulinum toxin therapy compared to that presented in the first edition of this book.

I would like to express my gratitude to Dr Fattaneh Tavassoli for her editorial assistance and to Drs Tahere Mousavi and Damoun Safarpour for their illustrations. I am also grateful to Merry Stuber and Charlotte Nunes from Springer Nature for their support and to Amrita Unnikrishnan who helped to finalize the printing of this book.

Newport Coast, CA, USA Bahman Jabbari
April 23, 2024

Contents

Chapter 1
A Toxin that Remedies a Large Number of Medical Problems: How It Happened?

Abstract This chapter provides information on the history of botulinum toxin development into a powerful therapeutic agent. It explains how through tireless efforts of remarkable basic scientists and clinicians one of deadliest toxins in the nature developed into a widely used treatment with high safety profile. Some of the characteristics of different brands of botulinum toxin in the US market are also briefly discussed in this chapter.

Keywords History of Botulinum toxin · Justinus Kerner · FDA approved indications of Botulinum toxins · Botulinum toxin · Botulinum neurotoxin

A group of bacterial toxins called botulinum toxins or botulinum neurotoxins (BoNT) have now become a remedy for a large number of hard to treat medical conditions. They have proven to be the most multipurpose therapeutic agents in modern medicine and possess more clinical applications than any other drug currently in the market [1]. Among these toxins, one type (type A) was first introduced to the medical arena in 1989 under the trade name of oculinum (name changed to Botox 2 years later). It was the only approved toxin in the US for several years. There are now several other type A botulinum toxins and a type B toxin as well, each with their advantages and disadvantages. Four decades of experience with botulinum toxin therapy indicates that these agents can be drugs of first choice for the symptoms of several medical conditions, and when used by trained clinicians, are generally safe. Serious side effects are rare, and in most cases gradually subside if the affected patients are diagnosed early and medically supported.

Botulinum neurotoxin, often abbreviated in the medical literature as BoNT, is produced by a bacterium with the medical name of clostridium botulinum (CB). The bacteria, CB, is present in nature and improper exposure to it can cause a disease called botulism. The term botulinum comes from the Latin word of "botulus" meaning sausage since the earlier (eighteenth and early nineteenth century)

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outbreaks of botulism in Europe were often linked to consumption of spoiled sausage or ham. The agent can get into the body and cause disease via a variety of routes: food consumption, inhalation, wound contamination and injection. In western countries, botulism is rare due to proper food preparation, wound hygiene, and protective laboratory regulations (to prevent inhalation toxicity). Botulism through therapeutic injections is also rare as the applied units of the toxin for most indications are below 500 units which is far from the lethal dose of 3000 units or more reported in monkeys [2]. Moreover, with modern and advanced life support facilities, even sick patients with respiratory failure, if diagnosed early, often eventually recover as the paralyzing effect of the toxin will not last more than 3–4 months.

The development of a therapeutic utility of botulinum neurotoxin (BoNT) took over a 100 years of clinical observation and laboratory experimentation. For years, physicians in Europe, especially in southern Germany, were familiar with the symptoms of a disease which was caused by consuming rotten sausage "sausage poisoning." After a well- documented outbreak of sausage poisoning in 1793 that affected 13 individuals (6 of whom did not survive), the city of Stuttgart became the major center for investigation of this type of poisoning [3]. At the beginning of the nineteenth century, a leading point of debate was whether the "sausage poisoning" was due to a chemical agent in the sausage or due to a biologic, yet unknown, factor. Several chemical agents were suspected including hydrocyanic acid.

The next major development was the prediction that the agent responsible for "sausage poisoning" could be used for treatment of symptoms of certain medical ailments. The individual who first promoted the idea was a young German physician, 29 years of age at the time, who studied in detail the latest outbreaks of the illness in southern Germany. Justinus Kerner (Fig. 1.1) published two monographs in 1820 and 1822 detailing the clinical aspects of botulism based on case histories of 76 and 155 patients [4]. Kerner's descriptions included almost all manifestations of botulism, as known to us today, including paralysis of the muscles, loss of pupillary reaction to light and diminished sweat and saliva production. After studying all ingredients of the poisoned food, he concluded that something in the fatty portion of the sausage itself and not any other ingredients in the sausage preparation (blood, liver, etc.) was responsible for the sickness. Kerner believed that this "fatty poison" had a biological rather than a chemical origin. He wrote that the toxic agent had to travel through the nervous system to cause paralysis and the other symptoms of the disease. The toxin damaged the nerves and made them like "rusted electrical wires".

Kerner predicted that the "sausage poison" could be used in the future to remedy certain symptoms of some medical disorders, particularly those symptoms arising from hyperexcitability of the nervous system that cause abnormal involuntary movements. He mentioned treatment of the involuntary movement of "chorea" as an example. Chorea is a movement disorder characterized by involuntary twitches which can affect the face or the limbs. Chorea may be hereditary (i.e. Huntington's chorea, often associated with dementia) or it may develop secondary to non-hereditary diseases or drugs. Currently, almost 200 years after Kerner's prediction, medicinal botulinum toxin injections have become the therapy of first choice for several abnormal movement and motor disorders especially dystonias and

Fig. 1.1 Portrait of Justinus Kerner from Wikimedia (public domain)

spasticity. Interestingly, however, it is least used in management of chorea—the movement disorder that he used as an example.

In 1895, Emile Van Ermengem, a professor of bacteriology at the University of Ghent, Belgium discovered the organism responsible for botulism (Fig. 1.2).

In 1919, Stanford university researcher A. Bruke discovered two different serological strains of BoNT, A and B. In 1924 Ida Bengston a Swedish -American bacteriologist suggested to change the name of bacteria from bacillus botulinum to clostridium botulinum. The word colstrididium is derived from Greek word of kloster meaning spindle.

Further refinement of the botulinum toxin which ultimately facilitated its clinical use, came about during World War II when there was an interest in producing large amounts of the toxin and to find preventive and therapeutic measures in case of exposure and intoxication. Close to the end of World War II, at Fort Detrick Maryland, a US Army research facility, Carl Lamanna and James Duff invented a technique for crystallization and concentration of botulinum toxin [2]. Edward Schantz (Fig. 1.4), purified and produced the first batch of the toxin in 1946. Shantz then moved to the University of Wisconsin where with Eric Johnson further refined botulinum toxin for clinical research.

In 1949, a British investigator, A. Burgen, and his colleagues discovered that botulinum toxin blocks the nerve transmitter substance “acetylcholine” at nerve-muscle junction leading to the toxin’s paralytic effect. In 1964, Daniel Drachman at Johns Hopkins University demonstrated that injection of the type A botulinum

Fig. 1.2 Emile Van Ermengem who discovered the culprit bacteria responsible for botulism. He studied the rotten ham consumed by a group of 34 musicians who all felt sick after an outgoing. He showed that the spoiled ham and the tissue obtained from 3 patients who did not survive, contained a large number of rod–shaped, gram positive bacteria which he named bacillus botulinum (Fig. 1.3)

neurotoxin (BoNT) into the muscles of chick embryos can produce a dose dependent muscle wasting (atrophy) and muscle weakening [5].

The next major step started with the work of Alan Scott and his colleagues in San Francisco, CA. Since early 1960s, Alan Scott, an ophthalmologist, and his colleague Carter Collins were interested in the physiology of eye muscles and correction of strabismus (crossed eyes) in children by a method other than resection of hyperactive muscles around the eye. At the time, their research focused on injection of anaesthetic agents into eye muscles of monkeys under electromyographic guidance. Electromyography records the electrical activity of muscles using a special instrument. Coming across Drachman's work, Dr. Scott started to explore the effects of botulinum toxin injections into the eye muscles of the monkeys. Edward Schantz who was then at University of Wisconsin, provided the purified and injectable toxin for Dr. Scott's experiments. In Scott's laboratory, the toxin was freeze-dried, buffered with albumin and prepared for injection in small aliquots.

In 1973, Dr. Scott published his seminal work on injection of botulinum toxin type A into the external eye muscles of monkeys. The work clearly showed that the toxin injection can selectively weaken a targeted eye muscle and offer an alternative to surgery for strabismus (crossed eyes). His subsequent work on 67 patients with strabismus (under an FDA approved protocol), published in 1980, demonstrated that indeed botulinum toxin injection was effective in correcting human strabismus by decreasing the overactivity of culprit eye muscle(s) and correctly aligning the two eyes [6]. Dr. Scott also showed, in a number of open label, unblinded small studies, that injection of botulinum toxin into face muscles of humans can slow

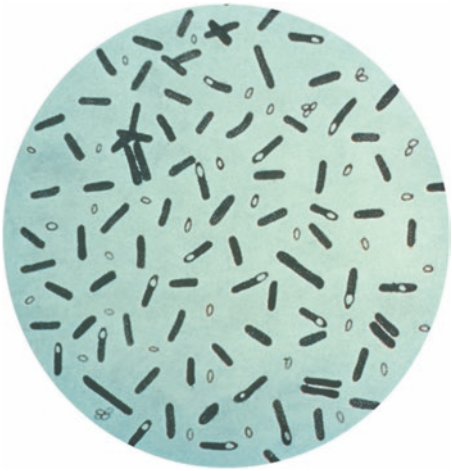

Fig. 1.3 Bacteria responsible for botulism. (Courtesy of Wikipedia)

Fig. 1.4 Edward Shantz and Eric Johnson in the laboratory at the University of Wisconsin. (From Dressler & Roggenkeaemper. Reproduced with permission from Springer)

down and even stop involuntary facial movements in conditions like blepharospasm (spasm of the eye lids) and hemifacial spasm (HFS) (involuntary contractions affecting half of the face). These observations ignited substantial interest among Movement Disorder specialists and consequently led to documentation of the efficacy of BoNT therapy for a large number of involuntary movements. Finally, Scott observed that injection of 300 units of Botox for treatment of spasticity (tense muscles with increased tone) in one setting did not cause any side effects. This observation indicated a margin of safety per single injection of botulinum toxin type-A (Botox) in human which was unknown prior to his report [7] (Fig. 1.5).

Dr. Scott's efforts along with the work of Stanley Fahn and Mitchell Brin at Columbia University of New York, Joseph Janckovic at Baylor Medical College and Joseph Tsui at the University of British Columbia led to the approval of botulinum toxin A (then called oculinum, marketed by Allergan- the name was changed

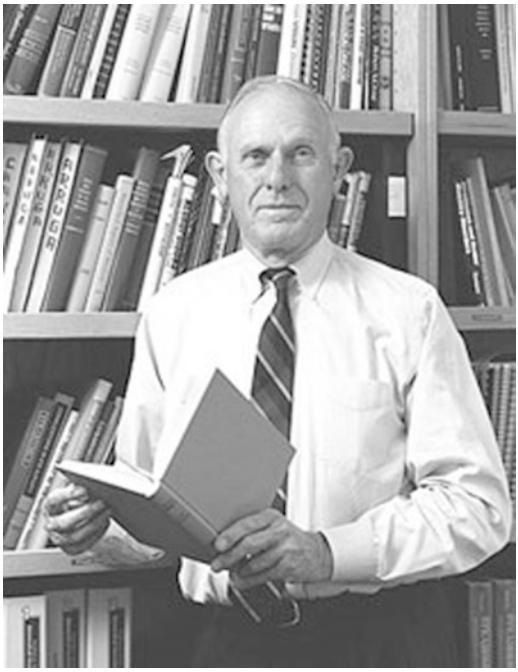

Fig. 1.5 Alan Scott who pioneered the use of botulinum neurotoxin (BoNT) therapy in humans. (From FJ Erbguth in the J Neural Trans. Reproduced by permission from Publisher-Springer)

to Botox 2 years later) for treatment of strabismus, blepharospasm and hemifacial (HFS) in 1989. The path was now open for investigation of the effects of BoNTs in many other movement and motor disorders.

What happened over the next 42 years is one of the most amazing stories in the field of medical treatment. A potent bacterial toxin which was the cause of much fear and apprehension developed into a therapeutic agent with documented or highly suggestive efficacy in alleviating more than 50 different medical symptoms. It was found to be generally safe if used with proper techniques of injection and under appropriate dosing guidelines. Much was learned during these years about the molecular structure of botulinum toxins [8], and their mechanism of action(s) on the nerve-muscle junction (Chap. 2), glandular tissue [9], and even pain pathways [10].

A few years after introduction of Botox, two more BoNT type-As were developed and subsequently marketed in the US under the trade names of Xeomin and Dysport. In Europe, Dirk Dressler, Reiner Benecki, Keith Foster and Andy Picket were leading investigators in defining the characteristics of these two newer forms of BoNTs and their potential for treating medical ailments [11]. A type B toxin was also marketed in the US under the trade name of Myobloc (Neurobloc in Europe). These BoNTs are now all FDA approved for different clinical indications. More recently, more type A toxins have been developed; one of them, Jeuveu received FDA approval in 2019 for aesthetic use and treatment of frown lines. Another type A toxin, Daxxify was approved in 2022 for aesthetic use and then in 2023 for

treatment of cervical dystonia. Over time, much was learned about the advantages and disadvantages of these newer toxins (detailed descriptions are provided in Chap. 3 of this book).

Encouraged by earlier promising results of Botox injection, investigators with innovative minds conducted careful, high quality, double blinded clinical trials. The results of these multicenter studies, conducted on a sizeable number of patients led to FDA approval of Botox for management of a variety of medical conditions. In 2002, FDA approved injections of Botox into the face for correction of wrinkles (Chap. 13) and, in 2004, FDA approved Botox for treatment and reduction of excessive sweating (hyperhidrosis) in the arm pit (axilla) (Chap. 14). In 2009, FDA approved Botox injections for treatment of a disabling movement disorder characterized by abnormal neck postures, neck pain and neck shakes (cervical dystonia-Chap. 8). In 2011 and 2013, Botox was approved for two types of bladder dysfunction causing urinary urgency and incontinence (neurogenic and hyperactive bladder (Chap. 10). As clinical research continued, the positive results of two large, multicenter studies (PREEMPT 1 and 2), showed the efficacy of Botox injections into the skin and muscles around the head in subjects with chronic migraine leading to FDA approval of this treatment in 2010 (Chap. 4). During the past 15 years, FDA approved Botox, Xeomin and Dysport for treatment of spasticity. Spasticity (stiff and tense muscles) is a major handicap for patient after stroke, head and spinal cord trauma and multiple sclerosis as well as children affected by cerebral palsy. Injection of botulinum toxin into the affected muscles reduces muscle tone, improves the limb function, and helps ambulation and physiotherapy (Chaps. 6 and 7).

In addition to these FDA approved medical indications, there are more than 20 other medical conditions that, according to the results of small blinded and quality studies, respond to botulinum toxin injections. There are strong suggestions that, BoNT injections into the skin can relieve several types of distressing pains such as pain associated with shingles, painful neuropathy due to diabetes or painful neuropathy secondary to trauma to the limb(s) [12] (Chap. 5). There is also compelling evidence that injection of BoNT into arm and forearm muscles can significantly reduce the hand tremor both in Parkinson disease and in essential tremor (Chap. 8) [13, 14]. This wide range of BoNT applications for treatment of different medical symptoms reflects multiple and diverse mechanisms of the toxin's action which is covered in more detail in the second chapter of this book. It is expected that continued medical research and clinical observations will further expand the indications of botulinum toxin therapy in clinical medicine.

Amid emerging clinical indications for botulinum toxin therapy, basic scientists started to explore specifics of botulinum toxin molecule and its mechanism of action in different medical disorders. In US, at Yale university (New Haven, CT), Professor James Rothman (Fig. 1.6), chairman of the Department of Cell Biology, defined special proteins (called SNARE) that work at synapses (where two nerve cells or a nerve cell and muscle cell connect with each other) and promote the release of a specific chemical at the synapse that conveys the nerve signal from one cell to another. His lab purified SNARE proteins, one of which is blocked by the function of Botox.

Fig. 1.6 Professor James Rothman, chair of Cell Biology at Yale who won the Nobel Prize in physiology and medicine for his seminal works on the physiology of synapses

Professor De Camilli and his colleagues, also at Yale, identified the protein that is blocked at neuro-muscular junction after Botox injection as SNAP-25 (Chap. 2) [15]. Italian basic scientists Monteccuco, Rossetto, Pirazzini described detailed molecular structure and pharmacology of botulinum toxins as well as the similarity and dissimilarity of this toxin to the tetanus toxin [8, 16]. In Zagreb, Croatia, Zdravko Lackovic, chairman of the Department of Pharmacology and his colleagues, Ivica Matak and Lidjia-Back-Rojecky have shown in a series of elegant experiments compelling evidence for the central action of the botulinum toxins and offered explanations on how the toxin influences pain pathways [17, 18].

Further evidence for the central function of botulinum toxins was provided by Matteo Caleo and his Italian colleagues after finding parts of the toxin in the central nervous system (brain) following injection into the muscle [19]. Gianpietro Schiavo in London, in collaboration with Italian scientists, discovered the enzyme through which botulinum toxins deactivate synapse proteins [20]. Professor Oliver Dolley and his colleagues in Dublin, Ireland have illustrated the mechanisms through which the injected toxin (into muscle) gets through different nerve cells and more recently how the novel recombinant toxin targets sensory cells and alters these cells' functions, an important finding pertaining to the analgesic effects of the toxin [21]. These remarkable achievements in the field of basic science, constantly encourages the clinicians to find new medical indications for botulinum toxins. Table 1.1 demonstrates important time-lines in the history of botulinum toxins from discovery to clinical application.

Table 1.1 Important time-lines of botulinum toxin development for clinical use

Year(s)	Investigator(s)/ FDA approvals	Comment
1820–1822	Justinus Kerner	Described details of botulism; predicted that the toxin could be used in the future as a medical remedy
1895	Emile Van Ermengem	Discovered the bacteria causing botulism
1944–1946	Lamanna and Duffy	Concentrated and crystalized the toxin
1946	Edward Schantz	Purified and produced the toxin in a form suitable for medical research
1949	A. Burgen	Acetylcholine identified as the chemical blocked by BoNT at nerve muscle junction
1953	Daniel Drachman	Intramuscular injection of Schantz's toxin can be quantified and resulted in dose dependent muscle weakness in chicks
1973	Alan Scott	Injection of type A toxin improved strabismus (crossed eyes) in monkeys
1980	Alan Scott	Controlled human study showed efficacy in strabismus. Observations made on potential use for blepharospasm, hemifacial spasm, spasticity
1985–1988	Fahn, Jankovic, Brin, Tsui	Controlled and blinded studies showed efficacy in blepharospasm and cervical dystonia
1989	FDA approval of Type A toxin (oculinum- name later changed to Botox)	Toxin approved for use in blepharospasm, hemifacial spasm and strabismus
1989-present	Other approved indications by FDA	Toxin approved for facial wrinkles, frown lines, cervical dystonia, chronic migraine, bladder dysfunction, upper and lower limb spasticity, excessive sweating of the arm pit and excessive drooling

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Chapter 2
Structure and Mechanism of Function of Botulinum Neurotoxins: How Does the Toxin Work

Abstract This chapter discusses the molecular structure of botulinum toxin and how the toxin gets into the nerve cells after being injected into the muscle or skin. It describes the sequence of events that occurs inside the muscle or nerve cell that lead to the beneficial effects of the toxin upon the nerve and muscle cells as well as sweat and saliva glands in the body.

Keywords Botulinum toxin · Botulinum neurotoxin · Botulinum toxin molecule · Botulinum toxin mode of action

Introduction

Botulinum toxin or botulinum neurotoxin (BoNT) is a protein produced by certain bacteria named clostridium botulinum (CB). The term clostridium refers to the shape of the bacteria which is spindle/rod shaped, and the term botulinum is derived from the Greek word of "botulus" meaning sausage. The name stems from earlier outbreaks of botulism in Europe (Germany in particular) that were caused by consumption of rotten sausage. The history of early botulism outbreaks, discovery of the responsible agent for botulism, purification and production of the botulinum toxin for medical research as well as early clinical trials with this toxin which led to the discovery of BoNT's effectiveness in treatment of medical disorders are presented in detail in Chap. 1. This chapter explains how this toxin works and how it can be used in different medical conditions.

The results of animal research and early human observations published in 1960s and 1970's, indicating a therapeutic potential for BoNT, encouraged basic scientists to explore the molecular structure of the toxin and its mode of action. Over the past 55 years, the efforts of basic scientists deciphered the exact molecular structure of BoNT and provided a substantial amount of knowledge about how the toxin molecule reaches the nerves and exerts its therapeutic action after peripheral injection (into muscle or skin).

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Botulinum toxin is structurally a protein with perfect machinery to exert its function through a set of well–defined mechanisms inside the nerve cell. There are 8 distinct types of botulinum toxins (A, B, C, D, E, F, G, X) that are structurally similar with only minor differences. Types A, B, E and F can cause botulism in human, whereas, types C and D cause botulism in domestic animals [1]. The type X toxin has been discovered relatively recently. The type X toxin has low potency and therefore does not seem to be useful for clinical use [2]. Recently, several subtypes of BoNT-A and BoNT-B have been discovered (A1…, B1…) [3–5]. Continued research efforts are underway to define the role of these subtypes. Currently, only types A and B (A1 and B1) are considered suitable for clinical use.

Botulinum toxin molecule (type A) is an approximately 900 KiloDalton (KD) complex which consists of a core toxin (150 KD) and a complex of surrounding proteins (>700 KD). Dalton, the unified atomic mass unit, is a standard unit that quantifies mass on an atomic or molecular scale. The surrounding proteins of the core toxin protect the toxin from being degraded in a hostile environment such as stomach acid after its ingestion. That is why high doses of the toxin presented in infected food resist stomach acid and, after absorption in the gut, cause botulism. However, when the BoNT is injected into a muscle, the tissue enzymes (protease) quickly separate the toxin from the surrounding proteins by a process termed "nicking." The core toxin molecule then reaches its target at nerve endings probably via the blood or lymphatic system [6].

The point where a nerve connects to a muscle is called neuromuscular junction. The point where two nerve cells connect or a nerve cell connects with a muscle cell is called synapse. At both neuromuscular junction (nerve muscle synapse) and at nerve cell to nerve cell synapse, the transfer of nerve impulse to the muscle or to another nerve cell requires presence of a special chemical called neurotransmitter. There are many neurotransmitters in the brain, but the neurotransmitter at neuromuscular junction is called acetylcholine. At neuromuscular junction, there is a membrane on the nerve side (nerve that reaches the muscle) and a membrane on the muscle side with a cleft in between (synaptic cleft). The nerve ending close to the muscle (neuromuscular junction) contain many small vesicles (small pouches) that contain the neurotransmitter acetylcholine (Fig. 2.1). When the nerve's electrical signal reaches the nerve ending, these vesicles rupture and pour their neurotransmitter contents into the cleft between the nerve and muscle membranes. The neurotransmitter (in this case acetylcholine) then attaches itself to the muscle membrane and activates the muscle. The injected botulinum neurotoxin (BoNT), by preventing acetylcholine release, can relax, weaken or even paralyze the muscle (depending on the dose). This is the mechanism by which the injected BoNT into the muscle improves stiffness of the muscles after a stroke, brain trauma or in children with cerebral palsy. The mechanism through which BoNT exerts its effect on nerve-muscle junction is complex and requires some knowledge of the core toxin's molecular structure [7–11].

Each molecule of the toxin consists of two structures, called light chain (designated as L) and heavy chain (designated as H). The molecular weight of light and heavy chains is 50 and 100 KD, respectively. KD stands for kilodalton. Dalton is the

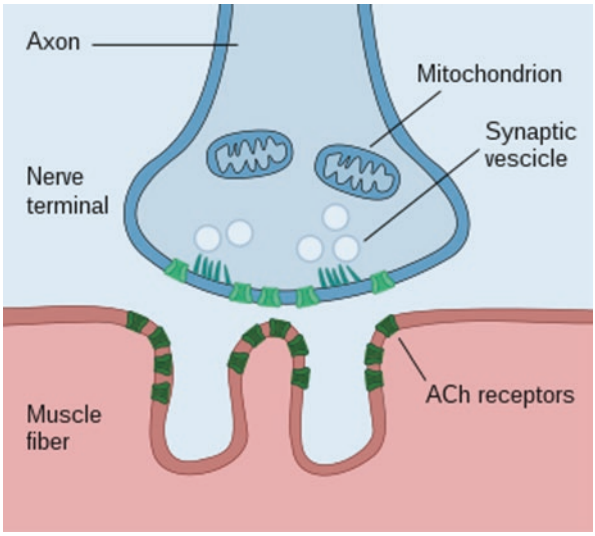

Fig. 2.1 Neuromuscular junction (NMJ): Nerve, nerve terminal, muscle fiber, and the cleft between. Nerve terminal shows vesicles that contain acetylcholine (ACH). Nerve signals reaching the nerve terminal at NMJ cause the rupture of the vesicles and release of acetylcholine into the synaptic cleft. ACH molecules attach to the muscle receptors on the surface of the muscle and activate the muscle. (From Wikipedia reproduced under Creative Commons Attribution-Share Alike 4.0 International license)

unit of atomic weight. The light (L) and heavy (H) chains are connected by a disulfide bond (ss) (Fig. 2.2).

The light chain is the catalytic domain of the toxin and its active moiety after entering the nerve cell. The heavy chain has two parts called HC and HN domains (Fig. 2.2). The HC domain (binding domain) attaches the toxin to the membrane receptors of the nerve cell. There are specific receptors on the nerve cell membrane that the HC domain of the toxin can attach itself to. The receptor for type A toxin is a protein called SV2. For type B toxin, two receptors have been identified. One is a complex sugar called ganglioside and the other is a protein called synaptogamin. After the toxin attaches to the receptor, the receptor undergoes structural modification and ends up working like a channel letting the toxin to go through. After entering the nerve terminal, the disulfide bond of BoNT breaks inside the nerve cell (via action of heavy chain -translocation) and the two chains (light and heavy) of the toxin separate from each other (Fig. 2.3).

The light chain (active moiety of the toxin that has the enzyme protease) is now free to exert its effect and prevent the release of acetylcholine from the synaptic vesicles [12–17]. It does this by attaching itself to specific synapse proteins whose function is to promote the fusion of the vesicle onto the nerve cell membrane. Fusion of the vesicle to cell membrane leads to vesicle rupture and release of acetylcholine into the synaptic cleft. The synapse proteins that promote vesicular fusion and vesicle rupture are called SNARE Proteins.

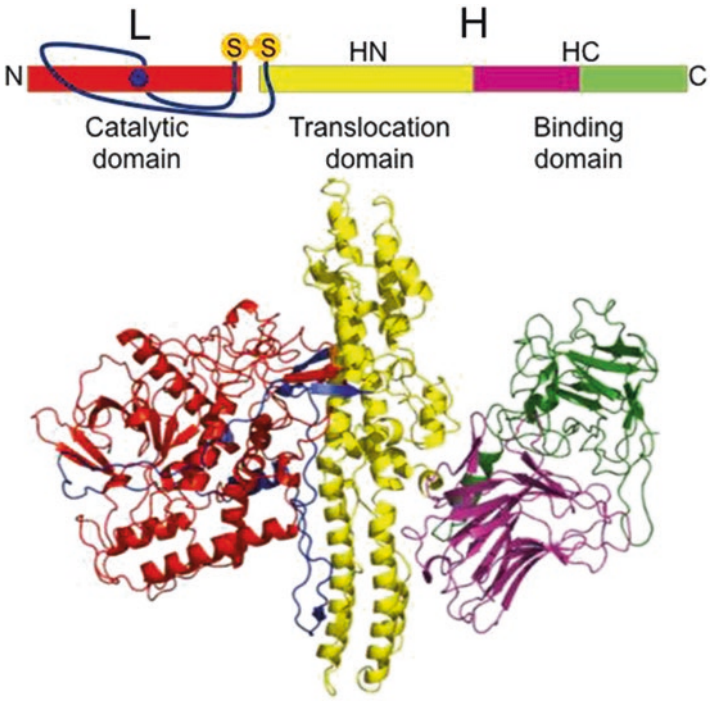

Fig. 2.2 Molecular structure of botulinum toxin. (From Rossetto O and co-workers 2014 reproduced with permission from publisher Nature Portfolio (Springer Nature))

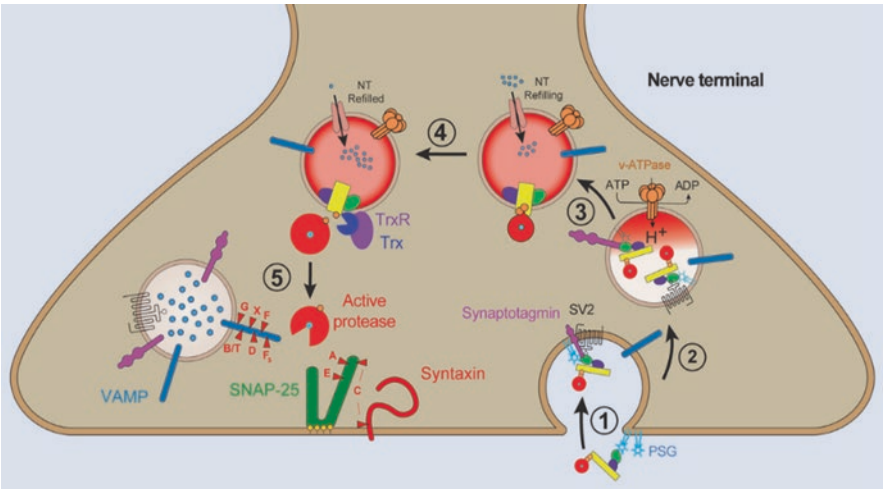

Fig. 2.3 Mechanisms of action of BoNTs. (From Rossetto et al. [10]. Reproduced with permission of publisher (Springer))

Over the past 40–50 years, a group of cell biologists succeeded to determine the mechanisms of vesicle fusion and synaptic machinery including the function of SNAREs [17–20]. Most notable among these scientists are J. Rothman, R. Schekman and TC. Südhof who won the Nobel prize in Medicine & Physiology in 2013 for their work in this area, when inside the nerve terminal and detached from the heavy chain, the light chain of the BoNT attaches itself to a specific SNARE that relates to a specific type of BoNT (for instance type A or B). After attachment to the SNARE protein the light chain of the toxin deactivate the SNARE protein via light chain's enzymatic function (a zinc activated protease). The result is inhibition of release of the neurotransmitter from the vesicle and, in case of nerve-muscle synapse, relaxation, weakness or even paralysis of the muscle depending on the dose of the injected toxin. The SNARE for type A toxins (Botox, Xeomin, Dysport) was first discovered by a group of Yale investigators and named SNAP 25 [21]. It is attached to the membrane of the nerve terminal. For the Type B toxin, the SNARE is attached to the vesicle wall itself and is designated as VAMP/Synaptobrevin (Fig. 2.3). The sequences of botulinum toxin's travel and activation after peripheral injection is presented in Table 2.1.

The binding of the BoNTs (A and B) to the nerve terminal is a long-term binding, that in case of nerve-muscle junction lasts for 3–4 months [22]. This long period of binding is medically desirable. For instance in spastic and tense muscles of patients with stroke or children with cerebral palsy, one injection could maintain the muscle relaxation for the entire period of binding (usually 3–4 months). Over time, the nerve ending starts to sprout and the new endings make contact with different muscle fibers. Finally, when the binding is over, the synapse resumes its full function. This is different from what happens to synapses in certain disease conditions (for instance ALS) where neurodegeneration leads to permanent loss of synapse function.

Table 2.1 Sequence of Botulinum toxin's action after injection into the muscle

1.	After injection into the muscle, protease, an enzyme inside the muscle separates the core toxin from protective proteins around it
2.	The released toxin molecule reaches nerve muscle junction probably via blood or lymphatic system
3.	The heavy chain of the toxin attaches the toxin molecule to certain receptors on the surface of nerve ending (SV2 for Botox)
4.	Receptors open as a channel and let the toxin molecule enter into the nerve terminal
5.	The disulfide bond of the toxin breaks inside of the nerve terminal via function of the heavy chain
6.	Freed light chain of the toxin (active or catalytic moiety) reaches the SNARE proteins and deactivates them via its enzymatic protease function
7.	Deactivation of SNARE protein prevents rupture of synaptic vesicles and release of acetylcholine
8.	Muscle deprived from acetylcholine activation relaxes and slightly weakens, an effect that improves muscle spasms, abnormally high muscle tone (spasticity) and involuntary movements

There is now substantial evidence that BoNT injected into the muscle after reaching the nerve terminal does not stay in the peripheral nerve and part of the molecule of BoNT travels to the central nervous system. The extent of central travel of the toxin is still under investigation. Nevertheless, convincing research evidence exists that a part of toxin molecule reaches the spinal cord and lower part of the brain (brain stem) after injecting the toxin into the muscle [23–25]. It is believed by many researchers, that this central travelling of the toxin may also help modulation of the motor function in a good way. The results would be improvement of motor function for example in spasticity (increased muscle tone) seen in adults with stroke or children with cerebral palsy. Treatment of spasticity with botulinum toxin injection is now approved by FDA for both adults and children and is one of the largest areas of BoNT use in clinical medicine [26–40].

Excessive Sweating and Drooling

Acetylcholine is also the neurotransmitter for the sympathetic nerve endings that supply nerves to sweat and salivary glands. BoNT injections into and under the skin in the areas where these glands are located such as arm pit, palm of the hands or bottom of the feet can significantly reduce sweating and help the affected individuals. Excessive hand sweating can be embarrassing during hand shaking; in case excessive sweating of the arm pit it can cause social discomfort. It many cases excessive sweating is genetic and runs in the family. Excessive drooling is also a nuisance and can be seen in Parkinson's disease or in children with cerebral palsy or individuals (children or adults) with brain damage. The glands that secrete saliva are located close to the angle of the jaw (parotid and submandibular) and are easily accessible from the surface by a thin and small needle. The injected BoNT (A or B) suppresses the excessive salivation within days, an effect that could last for 6 month. Injections are performed with a thin needle and cause minimal discomfort. A sizeable literature indicates efficacy of BoNT therapy for hyperhidrosis and hypersalivation (excessive sweating and excessive salivation) and attests to the safety of this form of treatment [41–52]. The use of BoNT for excessive sweating and excessive secretion of saliva is discussed in detail in Chap. 14 of this book.

Pain

Another important set of chemical neurotransmitters that are affected by peripheral injection (into muscle or skin) of BoNTs is pain neurotransmitters. The pain neurotransmitter(s) is not acetylcholine (in contrast to the muscle and gland) but other transmitters that are present in sensory nerves and in spinal cord or brain. These transmitters convey the unpleasant sensations from periphery to the brain. When they have sufficient intensity they are perceived as pain. Several pain

transmitters have been discovered and studies over the past 60–70 years; the most well known among them are glutamate, substance P and Calcitonin Gene Related Peptide (CGRP) [53–57]. Over the past 25 years, animal research have shown that peripheral injection of botulinum neurotoxins A or B can significantly decrease the activity of glutamate, substance P and CGRP in peripheral nerves and in the central nervous system (spinal cord and brain) [58–67].

The animal data demonstrating that peripheral injection of BoNTs can suppress the function of pain transmitters provided grounds for clinical researches to study the role of botulinum toxin therapy in human pain. The first breakthrough came after two large multicenter studies showed efficacy of Botox injections in chronic migraine (see Chap. 4 for detailed information of these studies, recommended sites of injection and applied doses) [68]. Subsequent studies have shown that BoNTs are effective in a number of other pain syndromes as well [69] (Chap. 5).

Over the past 20 years, additional data from animal studies and human observations suggest a "central" mechanism for the action of botulinum toxin molecules in pain disorders. The support for a central (spinal cord and possibly brain) mechanism of action for alleviation of pain after botulinum toxin's injection comes from several lines of research some example of which are described below:

1. In laboratory animals, direct application of BoNT to dura matter (the thin sheet of tissue that covers the brain) alleviated facial pain and reduced the inflammation of the dura caused by experimentally induced facial pain (ligation of a facial nerve) [70].
2. In an animal model of leg pain caused by diabetic neuropathy (nerve damage due to diabetes), injection of BoNT into one leg, not only reduced the pain in that leg but also in the other leg implying an analgesic function through a spinal cord loop (central effect) with participation of spinal cord nerve cells [71].

Much of the seminal works in animal studies of pain are done by Dr. Zdravko Lakovic chairman of department of and Department of Pharmacology and his colleagues in University of Zagreb, Croacia (Fig. 2.4).

Fig. 2.4 Dr. Zdravko Lacovic whose laboratory provided significant information on how botulinum toxin injection inhibits pain in peripheral and central nervous system(brain and spinal cord). (Photo kindly given to the author by professor Lakovic)

These central mechanisms, however, do not seem to exert any deleterious effect on the spinal cord or brain (in doses approved for clinical use) since millions of patients who receive BoNT injections every year do not complain of any untoward side effects related to central nervous system (seizures, memory loss, specific central motor disorders, etc.).

Recently, scientists have succeeded in making a toxin molecule consisting of combination of two toxins (chimera—for instance for instance E/A toxins), that can specifically target the sensory nerve cells and, hence, specifically treat pain [72–74]. Limited number of studies have shown that in animal models, these toxin chimeras can suppress experimental pain [75]. The effect of these toxin chimeras in human pain is currently under investigation.

The details of botulinum neurotoxins' biology, pharmacology, and toxicology can be found in a recently published comprehensive review [76].

Hyperactive Involuntary Movement Disorders (HIMD)

Botulinum toxins are now widely used for treatment of HIMDs. Most notable examples include cervical dystonia, hemifacial spasm and blepharospasm, three indications that have received early FDA approval for use in the US. Over the past 40 years, experience with botulinum toxin therapy for these movement disorders had been very positive; toxin therapy has now been established as treatment of first choice for these conditions [77, 78]. In the experienced hands using recommended doses, botulinum toxin therapy has proved to be safe and serious side effects are rare and preventable (see Chap. 17 of this book on safety issues).

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