Chapter 1
The History of Botulinum Neurotoxins: From 1820 to 2020

Bahman Jabbari

Abstract Nearly 200 years ago (1820), a young German physician Justinus Kerner predicted that the agent responsible for "sausage poisoning "could have therapeutic implications. The agent *Clostridium botulinum* was discovered at the end of the nineteenth century by the Belgian bacteriologist Emile Van Ermengem. Close to end of World War II, the toxin was isolated and purified by Lamanna and Duff and was prepared and produced for clinical use by Schantz. Allen Scott, following a series of studies in monkeys, published the first utility of botulinum neurotoxin (BoNT) in humans for correcting strabismus in 1980. The past 40 years witnessed the development of vast clinical indications of botulinum neurotoxin (BoNT) therapy. This chapter, in addition to the older historical data, also briefly discusses the contribution of some of contemporary basic scientists and clinical neurotoxicologists who are responsible for the therapeutic success of BoNT therapy in medical and surgical fields.

Keywords History of botulinum toxin · Sausage poisoning · Justinus Kerner · Van Ermengem · Allen Scott

Introduction

Nearly 200 years has passed since 1820 when Justinus Kerner published the first comprehensive account of botulinum toxin intoxication, then known as sausage poisoning. The part of southern Germany where Kerner was born and practiced medicine, Swabia (now Bavaria and Baden-Wurttemberg) had been experiencing outbreaks of "sausage poisoning" during the second half of the eighteenth century. These outbreaks increased during the Napoleonic Wars (1796–1813) when the ravished area suffered from poverty and smoking the sausage was performed under poor hygienic conditions. The issue of sausage poisoning was discussed during

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Fig. 1.1 Swabia with its capital Augsburg located between Munich and Stuttgart

these years in the Department of Medical Affairs of Kingdom of Wurttemberg several times seeking opinions from University professors in Tuningen and Stuttgart. A new outbreak in 1815, during which three of seven intoxicated patients died, further put demands on the medical community of the region to find the cause and remedy of sausage poisoning.

Justinus Kerner (Fig. 1.1), as a young physician and a native of the land, took an interest in the issue and first published a brief account of this illness in 1817. His more detailed paper of 1820 was based on observation of 76 patients. In this paper, Kerner described nearly all major symptoms of botulinum toxin intoxication, as we recognize today such as muscle weakness, paralysis of eye muscles, difficulty in swallowing, dry mouth, and some other signs of autonomic dysfunction. He then reported a larger observation on 155 patients in 1922. This was followed by a series of in vivo animal experimentations including a brief experiment on himself where he noticed severe dryness of the mouth after placing a small fragment of a spoiled sausage on his tongue. He concluded from his experiments that the toxin, potentially lethal, develops in the spoiled sausage in anaerobic milieu and exerts its ill effect mainly upon the motor and autonomic systems, sparing the sensory system (Fig. 1.2).

Kerner was the first to suggest that the "fatty toxin" in the spoiled sausage could find medicinal use in the future, especially in the area of hyperactive (hyperkinetic) movement disorders due to its muscle weakening effect; as an example, he mentioned the involuntary movement of chorea. Kerner also believed that, contrary to the common belief of the time that the culprit in the spoiled sausage was a chemical (fatty acid, prussic acid), it was probably a biologic (zoonotic) toxin.

We owe much of the information about Kerner to the German medical historian FJ Erbguth who has researched and described in detail Kerner's medical accomplishments in a series of articles [1–3]. Kerner was also an accomplished poet and avid traveler who was considered by Hermann Hesse – the Nobel Laureate of

Fig. 1.2 Justinus Kerner 1786–1862. (From Erbguth reproduced with permission from Springer)

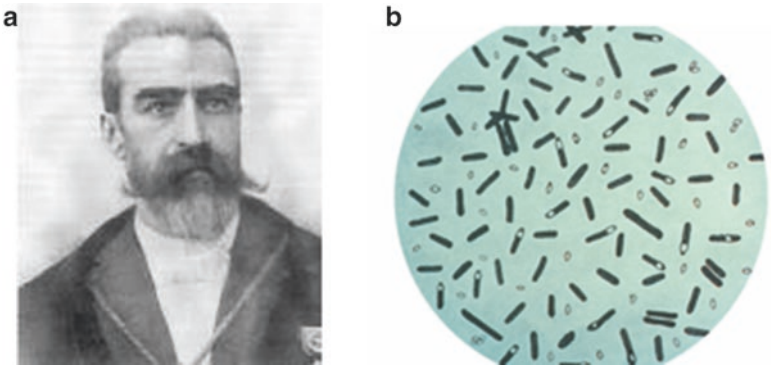

Fig. 1.3 (**a**) Emile Van Ermengem 1851–1932. (**b**) *Clostridium botulinum.* (From FJ Erbguth reproduced with permission from Springer)

Germany (1946) – as one of the three true German poets of his era. Interestingly, poisoning from blood sausage was recognized during the medieval era as well. The Byzantine emperor Leo the IV (750–780 AD) signed an order to stop the making and eating of blood sausage prepared in the pig stomach [4].

The next major event was the discovery of the responsible agent in 1895. On December 14, 1895, a group of 34 musicians who had attended a funeral became very sick and developed signs of botulism after consuming spoiled ham. Three of the 34 musicians died. The ham was sent to Emile Van Ermengem, professor of bacteriology at Ghent University, Belgium (Fig. 1.3a). Ermengem was able to produce similar signs of illness in animals after injecting them with the tissue containing the toxin. His microscopic examination revealed anaerobic Gram-positive, rod-shaped bacteria in the spoiled ham and tissue obtained from the dead musicians; he named the organism *Bacillus botulinus*, believing it to be the source of the culprit toxin in the ham (Fig. 1.3b).

In 1919, A. Burke from Stanford University published a paper on the serological types of botulinum toxins defining type A and type B toxins. In 1924, at the suggestion of Ida Bengtson, a Swedish-American bacteriologist, the name *Bacillus*

botulinus was changed to *Clostridium botulinum*. The word clostridium is derived from Greek word "Kloster," meaning spindle. The genus *Clostridium* includes a group of anaerobic bacteria such as *Clostridium tetani* responsible for the production of tetanus toxin.

During World War II, all parties were very interested to purify and develop botulinum toxin both as a weapon and to find measures to protect the soldier in case of exposure. Close to the end of the war, James Lamanna and Richard Duff working at Fort Detrick, Maryland, a US Army facility, discovered a technique to crystalize and concentrate botulinum toxin. In 1946, Edward Schantz, working at the same facility, purified and produced a large amount of the toxin. Schantz then moved to the University of Wisconsin where, in collaboration with Erik Johnson (Fig. 1.4), he further refined the toxin and made it available for clinical research.

In 1949, the British investigator A. Burgen and his colleagues discovered that the paralytic effect of botulinum neurotoxin is related to its effect on the neuromuscular junction via blocking the release of acetylcholine [5]. In 1964, Daniel Drachman at Johns Hopkins using Schantz's toxin demonstrated that injection of botulinum toxin A into the hind limb of chicken's embryo can cause a dose-dependent muscle weakness and atrophy [6]. His work came to the attention of Allen Scott (Fig. 1.5) and his colleague Carter Collins, ophthalmologists in San Francisco, who were interested in improving strabismus in children by methods other than surgery. At that time, their research focused on injection of anesthetic agents into monkey's eye muscles under electromyographic guidance.

For the next decade, Dr. Scott, borrowing botulinum toxin from Edward Schantz laboratory in Wisconsin, conducted a series of experiments in monkeys by injecting the toxin into the extraocular muscles. His seminal publication in 1973 showed that injection of botulinum toxin can weaken the eye muscle of the monkeys, and this selective weakening had the potential of improving strabismus. His subsequent important work published in 1980 on 67 patients under an FDA-approved protocol demonstrated that BoNT injection into selected eye muscles can indeed improve

Fig. 1.4 Edward Schantz and Erik Johnson. (From Dressler and Roggenkaemper, reproduced by permission from Springer)

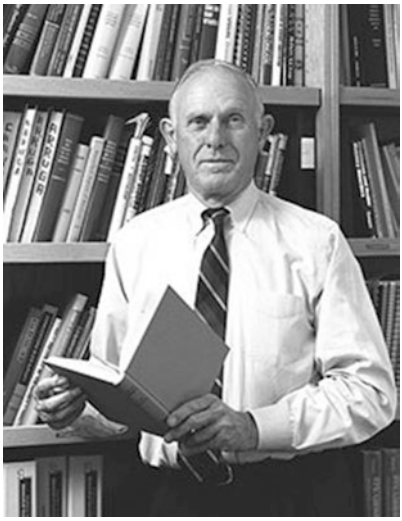

Fig. 1.5 Dr. Alan Scott who pioneered BoNT therapy in humans. (From FJ Erbguth reproduced with permission from Springer)

strabismus in human subjects [7]. During the 1980s in a number of small open label studies, Scott and his colleagues showed that injection of BoNTs into the face can improve hyperactive face movements such as blepharospasm and hemifacial spasm. Scott was also first to show that injection of 300 units of onabotulinumtoxinA (then called oculinum) in a single session (for spasticity) is safe, a safety margin that was not known prior to his observation [8].

These observations were of great interest to movement disorder specialists and led to conduction of several small blinded protocols by US (Fahn, Jankovic, Brin, and others) and Canadian (Tsui and others) investigators in the 1980s, ultimately resulting in FDA approval of botulinum toxin A in 1989 for blepharospasm, hemifacial spasm, and strabismus (based on Scott's work) (Table 1.1).

The initial name of oculinum used in earlier studies was changed to Botox, 2 years later, when Allergan Inc. acquired the right of the toxin distribution and marketing.

Along these clinical developments, our knowledge about the molecular structure of the toxin and where and how it works improved significantly through the tireless efforts of biologist and basic scientists; the contributions of some of them are described briefly at the end of this chapter (Fig. 1.6).

What happened next is one of the most amazing developments in clinical pharmacotherapy. A feared and lethal toxin was shown to be effective and relatively safe for treatment of a large number of medical and surgical conditions [9]. More recently, its use has been extended to the field of dentistry and veterinary medicine (Chaps. 16, 17, and 18 of this book). Other botulinum neurotoxins (incobotulinumtoxinA, abobotulinumtoxinA) and BoNT-B (rimabotulinumtoxinB) have found to be effective in several medical and surgical conditions as well. Newly developed botulinum toxins such as Korean toxin Meditox and Chinese toxin (Prosigne) have also shown promise in a number of neuropathic pain conditions [10]. A new form of

Table 1.1 Important timelines of botulinum toxin (BoNT) development for clinical use

Year	Investigator(s)/FDA approvals	Comment
1820–1822	Justinus Kerner	Describes details of botulism; predicted the toxin can be used in the future as medical remedy
1895	Emile Van Ermengem	Discovery of bacteria causing botulism
1944–1946	Lamanna and Duffy	Concentrated and crystalized the toxin
1946	Edward Schantz	Purified and produced the toxin in 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 Schantz's toxin can be quantified and causes dose-dependent muscle weakness in chicks
1973	Alan Scott	Injection of type A toxin improves strabismus in monkeys
1980	Alan Scott	Controlled human study showed efficacy in strabismus. Observations on potential use for blepharospasm, hemifacial spasm, spasticity
1985–1988	Fahn, Jankovic,Brin, Tsui	Controlled and blinded studies show efficacy in blepharospasm and cervical dystonia
1989	FDA approval of type A toxin (oculinum – name later changed to Botox)	Blepharospasm, hemifacial spasm, and strabismus
1989–present	FDA approved several other indications	Facial wrinkles, frown lines, cervical dystonia, chronic migraine, bladder dysfunction, upper and lower limb spasticity, axillary sweating

Fig. 1.6 Dr. James Rothman, Yale cell biologist who won the Nobel Prize in 2013 for his work on synapse physiology. His laboratory purified the SNARE complex

botulinum toxin A (prabotulinumtoxinA, Jeuveau) recently received approval for treatment of frown lines in the USA. Chapter 3 of this book describes the characteristics of available and marketed botulinum toxins as well as their similarities and differences. The list of US marketed botulinum neurotoxins, their clinical indication, and the year of FDA approval for each indication is presented in Table 1.2.

This brief account of botulinum toxin history will not do justice to the subject if it did not include significant contribution of recent contemporary basic scientists and clinical neurotoxicologists who have been instrumental for the current status of

Table 1.2 Clinical indications approved by FDA for botulinum toxins marketed in the USA

Generic and trade names	Abbreviation	Manufacturer	Approved indication (FDA)	Year of FDA approval
OnabotulinumtoxinA; Botox	OnaBoNT-A	Allergan Inc.; Dublin, Ireland	Blepharospasm Hemifacial spasm Strabismus Cervical dystonia Glabellar lines Axillary hyperhidrosis Chronic migraine Upper limb spasticity Neurogenic bladder Lateral canthal lines Overactive bladder Adult lower limb spasticity Forehead lines Pediatric lower and upper limb Spasticity	1989 1989 1989 2000 2002 2004 2010 2010 2011 2013 2013 2016 2017 2019
IncobotulinumtoxinA; Xeomin	IncoBoNT-A	Merz Pharma GmbH & Co; Frankfurt, Germany	Cervical dystonia Blepharospasm Glabellar lines Adult upper limb Spasticity Sialorrhea	2010 2010 2011 2015 2018
AbobotulinumtoxinA; Dysport	AboBoNT-A	Ipsen Pharmaceutical; UK	Cervical dystonia Glabellar lines Adult upper limb spasticity) Pediatric lower limb spasticity Adult lower limb spasticity Wrinkles	2009 2009 2015 2016 2017 2019?
RimabotulinumtoxinB; Myobloc/Neurobloc	RimaBoNT-B	US World Med-Solstice	Cervical dystonia Sialorrhea	2009 2010
PrabotulinumtoxinA Jeuveau	PraboBoNT-A	Evolus Inc.; Santa Barbara, CA	Frown lines	2019

BoNT therapy in 2020. Some of the most influential individuals in this field are discussed below. The list is by no means complete.

Biology and Basic Science

James E. Rothman, PhD, chairman of the department of cell biology at Yale University, revolutionized the field of cell biology by studying the molecular processes in a cell-free system. His work discovered many genetic and functional aspects of synapse physiology including vesicular trafficking, vesicular fusion, and proteins involved in this function. He identified genes and enzymes responsible for the budding of vesicles and their fusion with membranes. Dr. Rothman's laboratory succeeded in purification of the SNARE complex and provided pivotal evidence for establishing the central role of the SNARE complex (proteins targeted by botulinum toxins) in mediating membrane fusion. In 2013, Dr. Rottman was awarded the Nobel Prize in Medicine and Physiology.

Cesare Montecucco's first seminal work on BoNTs was the proposal of the double-receptor model in 1986 that is now well established for the majority of BoNTs. In 1992, he demonstrated that the common belief that the opposite symptoms of botulinum and tetanus toxins (flaccid versus spastic paralysis, respectively) are induced by different molecular actions is incorrect. In fact, the cleavage of a single protein is essential for the function of both toxins. The opposite symptoms are simply due to the different neurons targeted by tetanus and botulinum neurotoxins: the inhibitory interneurons of the spinal cord and the peripheral cholinergic neurons, respectively [11]. This was a major breakthrough in the understanding of the molecular pathogenesis of these diseases.

Gianmpietro Schiavo with a series of pioneering experiments, together with Cesare Montecucco, demonstrated that the inhibition of synaptic activity caused by tetanus and botulinum neurotoxins is due to a specific protease activity [12]. He showed that these neurotoxins cleave three synaptic proteins that play fundamental roles in neurotransmitter release. This discovery was instrumental for the field of SNARE biology and generated great interest worldwide. The seminal discovery of SNARE proteins as the substrates for BoNTs and TeNT in the early 1990s, led by Giampietro Schiavo and Cesare Montecucco, along with the groundbreaking work from James Rothman's laboratory on the purification of the SNARE complex, provided pivotal evidence for establishing the central role of the SNARE complex in mediating membrane fusion.

Matteo Caleo research on botulinum neurotoxins was devoted to their central effects. In collaboration with Cesare Montecucco in Padua and Gipi Schiavo in London, he demonstrated that BoNTs are retrogradely transported from the injected muscle along the axons of motoneurons and directly affect neurotransmission in central areas [13].

Ornella Rossetto's collaboration with Prof. Montecucco and Prof. Giampietro Schiavo led to the discovery of the zinc-endopeptidase activity of tetanus and

botulinum neurotoxins and provided initial experimental evidence that the molecular basis of their exceptional specificity is based on a double recognition of the substrate, i.e., of the cleavage site and of other regions outside the cleavage site termed SNARE motif.

Zdravko Lackovic, chairman of department of pharmacology in Zagreb, Croatia, along with his colleagues **Ivica Matak**, **Lidjia Back-Rojecky, and Boris Filipovic** through a series of elegant experiments, provided strong evidence for the central action of botulinum toxins in the pain pathways [14, 15]. Their findings have improved our knowledge about the central analgesic mechanisms of botulinum neurotoxins in pain. Their contributions to this field have opened the path and encouraged many clinical neurotoxicologist to conduct controlled clinical trials in different pain disorders.

Oliver Dolley, research professor and director of the International Center for Neurotherapeutics (ICNT) in Dublin, has done multidisciplinary investigations on the molecular basis of communications in the nervous system searching for proteins responsible for the fundamental process of transmitter release and its indirect regulation of voltage-sensitive K+ channels. His investigations have provided important information on endocytosis of botulinum neurotoxins by glutamatergic and peptidergic neurons. His most recent work has focused on selective targeting of sensory spinal cord by different agents to achieve analgesia. He has been successful in producing analgesia in rats by using a novel A/E toxin chimera [16].

Pietro De Camilli, MD, PhD

Dr. De Camilli is professor of neuroscience and cell biology at Yale University and founding director of the Yale Program in Cellular Neuroscience, Neurodegeneration, and Repair. His research has provided insight into mechanisms of membrane fission and has revealed ways through which membrane-associated proteins can generate, sense, and stabilize lipid bilayer curvature. His discovery and characterization of the role of phosphoinositide metabolism in the control of endocytosis have broad implications in the fields of phospholipid signaling and of membrane traffic. Dr. De Camilli and his collaborators were to first to discover that the synapse protein targeted by BoNT-A is SNAP-25 [17].

Neurologists: Clinical Neurotoxicologists in the USA

Joseph Jankovic, MD

Joseph Jankovic MD, professor of neurology at Baylor College of Medicine, is probably the most influential clinician/neuroscientist in discovering and promoting different clinical indications for the use of botulinum neurotoxins in medicine. He has contributed, often as a leader, in many well-designed clinical trials with botulinum toxins for different indications. His rating scale for blepharospasm is widely used specially in clinical trials of botulinum toxins. He is an outstanding teacher, who over the years has trained many fellows and young physicians for proficiency

in botulinum toxin treatment. As a prolific writer, his list of publication in Medline as of February 1, 2020, includes 177 articles on the subject of botulinum neurotoxins. Dr. Jankovic has held many important positions in national and international toxin-related forums. He is the recipient of lifetime achievement award at the international Toxin conference in 2019.

Mark Hallett, MD
Dr. Mark Hallett is Chief human motor control section in NINDS, National Institutes of Health at Bethesda, Maryland. As an internationally renowned figure in the field of movement disorders and clinical neurophysiology, Dr. Hallett has provided evidence that intramuscular injection of botulinum toxins changes the electrophysiology of muscle, peripheral nerves, and central nervous system. Under his watch, botulinum toxin treatment of movement disorders developed in NIH. Young and brilliant faculties such as Leonard Cohen, Barbara Illowsky Karp, Cordin Lungu, and Kathrine Alter developed expertise in different areas of their interest and rose to level of international experts in this field. His group conducted the most comprehensive studies of BoNT therapy in task-specific dystonias (Medline articles related to BoNTs: 60). He is the recipient of life time achievement award in international Toxin conference in 2017.

Michell Brin, MD
Dr. Brin was trained under Stanley Fahn, MD, at Columbia University, NY, and conducted some of the earliest studies of onabotulinumtoxinA efficacy in movement disorders (mostly tremor and dystonia). Over the past 30 years, he has been a key investigator in a large number of clinical trials. As an executive at Allergan Inc., he has been a key player in FDA approval of onabotulinumtoxinA for several clinical conditions (migraine, spasticity, cervical dystonia, axillary hyperhidrosis) (Medline articles related to BoNTs: 98).

Cynthia Comella, MD
Professor of neurosurgery and neurological sciences at Rush Medical School, Chicago, Ill, Dr. Comella has been a major contributor and investigator in several multicenter studies conducted on botulinum toxin therapy in the USA. Her major area of work has been on investigating the effect of botulinum toxins in cervical dystonia. Through her efforts and those of her collaborators, all four marketed BoNTs in the USA received approval by FDA for US use in cervical dystonia. As an expert electromyographer, Dr. Comella defined precise injecting methods to target difficult neck muscles in cervical dystonia. Her educational workshops in the annual meetings of the American Academy of Neurology are popular and well received (Medline articles related to BoNTs: 55).

David M. Simpson, MD
David M. Simpson, MD, FAAN, is professor of neurology at the Icahn School of Medicine at Mount Sinai, Department of Neurology. He is director of the Neuromuscular Diseases Division and the Clinical Neurophysiology Laboratories. His main area of toxin work focuses on the study of BoNTs in spasticity. He and his colleagues have shown, in an important study, that up to 800 units of

incobotulinumtoxinA in one session can be used for treatment of poststroke spasticity without serious side effects [18]. He is the chair of the Guidelines and Assessment Subcommittee of AAN that periodically assesses the efficacy of BoNTs for different neurological disorders (Medline articles related to BoNTs: 36).

Daniel Troung, MD
Dr. Truong has been a major contributor to multicenter studies in cervical dystonia and blepharospasm. His book *Manual of Botulinum Toxin Therapy* has been received with enthusiasm worldwide due to its practical points delivered with remarkable anatomical drawings. The book has been translated in many languages (Medline articles related to BoNTs: 40).

Bahman Jabbari, MD
Bahman Jabbari, emeritus professor of neurology at Yale University, started his practice and research on BoNT therapy in 1990 by establishing a comprehensive BoNT therapy clinic at Walter Reed Army Medical Center, Washington, DC, and 15 years later at Yale University School of Medicine in New Haven, CT. He and his colleagues were first to show the efficacy of BoNT therapy in plantar fasciitis and in nonsurgical low back pain. His most recent contribution is designing a special EMG-guided method that can significantly reduce the incidence of hand and finger weakness after BoNT injection into the forearm muscles of patients with Parkinson tremor and ET. Dr. Jabbari is the author of two books on botulinum toxin therapy and editor of two books on the same subject (Medline articles related to BoNTs: 45).

PREEMPT Group (Drs. Silberstien, Dodick, Aurora, Lipton, Blumenfeld, and Others)

A group of investigators, expert in treatment of headache, through two well-designed multicenter, blinded clinical trials (PEEMPT I and II), demonstrated the efficacy of onabotulinumtoxinA injections in chronic migraine [19] that led to its FDA approval in 2010. Subsequently, in a series of articles using the large PREEMPT cohort, they have shown that BoNT therapy in chronic migraine also improves the patients' quality of life and is effective in migraineurs with medication overuse. BoNT therapy is now an established treatment for chronic migraine worldwide.

Germany

Dirk Dressler, MD
Dr. Dressler, director of division of movement disorders in the University of Hanover, Germany, is probably the most influential clinical neurotoxicologist and clinical toxin researcher in Europe. He developed an interest in BoNT therapy

during his training with late David Marsden in National Hospital of London (the 1980s). He was the first person who organized BoNT therapy in Europe and is the individual with most clinical toxin-related publications in the European continent [20]. Dr. Dressler is the author of two books on botulinum toxin therapy, the first one in German and the second in English.

Reiner Benecke, MD
Dr. Benecke, like Dr. Dressler, developed his interest in clinical use of botulinum neurotoxins while working with Marsden's group in London. Dr. Beneke and Dr. Dressler participated in the development of incobotulinumtoxinA (Xeomin), then called NT201 (in research protocols), during their several years of partnership in Rostock, Germany. Their publications on merits of incobotulinumtoxinA as a BoNT free of neutralizing proteins and on immunology of botulinum neurotoxins paved the way for extensive use of this form of BoNT-A in Europe and the USA. The list of other German physicians with expertise in botulinum toxin therapy and significant contributions to this field include (but not limited to) Wolfgang Jost, Gerhard Reichel, Markus Naumann, Jorg Wissel, and Fereshteh Adib Saberi.

Austria

Werner Poewe, MD
Professor Poewe is the chairman of the Department of Neurology in Medical University of Innsbruck, Austria. He conducted several clinical trials assessing the efficacy of BoNTs in different movement disorders and spasticity. In an early study, he has shown that in children and young adults, 200 units of onabotulinum injected per leg is relatively safe and is more effective than a lower dose of 100 units. He served as president of the International Movement Disorder Society from 2000 to 2002 and as president of the Austrian Society of Neurology from 2002 to 2004. He is the author of a book entitled *Botulinum Toxin in the Treatment of Cerebral Palsy* (Medline articles related to BoNTs: 38).

References

1. Erbguth FJ. Botulinum toxin, a historical note. Lancet. 1998;351(9118):1820. https://doi.org/10.1016/S0140-6736(05)78793-6.
2. Erbguth FJ. Historical notes on botulism, Clostridium botulinum, botulinum toxin, and the idea of the therapeutic use of the toxin. Mov Disord. 2004;19(Suppl 8):S2–6. https://doi.org/10.1002/mds.20003.
3. Erbguth FJ. From poison to remedy: the chequered history of botulinum toxin. J Neural Transm (Vienna). 2008;115(4):559–65. https://doi.org/10.1007/s00702-007-0728-2.
4. Whitcup SM. The history of botulinum toxins in medicine: a thousand year journey [published online ahead of print, 2019 Aug 27]. Handb Exp Pharmacol. 2019; https://doi.org/10.1007/164_2019_271.

5. Burgen AS, Dickens F, Zatman LJ. The action of botulinum toxin on the neuro-muscular junction. J Physiol. 1949;109(1–2):10–24. https://doi.org/10.1113/jphysiol.1949.sp004364.
6. Drachman DB. Atrophy of skeletal muscles in chicks embryo treated with botulinum toxin. Science. 1964;145(3633):719–21. https://doi.org/10.1126/science.145.3633.719.
7. Scott AB. Botulinum toxin injection into extraocular muscles as an alternative to strabismus surgery. J Pediatr Ophthalmol Strabismus. 1980;17(1):21–5.
8. Scott AB. Development of botulinum toxin therapy. Dermatol Clin. 2004;22(2):131–v. https://doi.org/10.1016/s0733-8635(03)00019-6.
9. Jankovic J. Botulinum toxin: state of the art. Mov Disord. 2017;32(8):1131–8. https://doi.org/10.1002/mds.27072.
10. Jabbari B. Botulinum toxins in pain disorders: Springer Nature; New York 2015.
11. Schiavo G, Benfenati F, Poulain B, et al. Tetanus and botulinum-B neurotoxins block neurotransmitter release by proteolytic cleavage of synaptobrevin. Nature. 1992;359(6398):832–5. https://doi.org/10.1038/359832a0.
12. Schiavo G, Rossetto O, Santucci A, DasGupta BR, Montecucco C. Botulinum neurotoxins are zinc proteins. J Biol Chem. 1992;267(33):23479–83.
13. Caleo M, Spinelli M, Colosimo F, et al. Transynaptic action of botulinum neurotoxin type a at Central Cholinergic Boutons. J Neurosci. 2018;38(48):10329–37. https://doi.org/10.1523/JNEUROSCI.0294-18.2018.
14. Filipović B, Matak I, Bach-Rojecky L, Lacković Z. Central action of peripherally applied botulinum toxin type A on pain and dural protein extravasation in rat model of trigeminal neuropathy. PLoS One. 2012;7(1):e29803. https://doi.org/10.1371/journal.pone.0029803.
15. Matak I. Evidence for central antispastic effect of botulinum toxin type A. Br J Pharmacol. 2020;177(1):65–76. https://doi.org/10.1111/bph.14846.
16. Wang J, Casals-Diaz L, Zurawski T, et al. A novel therapeutic with two SNAP-25 inactivating proteases shows long-lasting anti-hyperalgesic activity in a rat model of neuropathic pain. Neuropharmacology. 2017;118:223–32. https://doi.org/10.1016/j.neuropharm.2017.03.026.
17. Blasi J, Chapman ER, Link E, et al. Botulinum neurotoxin A selectively cleaves the synaptic protein SNAP-25. Nature. 1993;365(6442):160–3. https://doi.org/10.1038/365160a0.
18. Dodick DW, Turkel CC, DeGryse RE, et al. OnabotulinumtoxinA for treatment of chronic migraine: pooled results from the double-blind, randomized, placebo-controlled phases of the PREEMPT clinical program. Headache. 2010;50(6):921–36. https://doi.org/10.1111/j.1526-4610.2010.01678.x.
19. Wissel J, Bensmail D, Ferreira JJ, et al. Safety and efficacy of incobotulinumtoxinA doses up to 800 U in limb spasticity: the TOWER study. Neurology. 2017;88(14):1321–8. https://doi.org/10.1212/WNL.0000000000003789.
20. Dressler D, Roggenkaemper P. A brief history of neurological botulinum toxin therapy in Germany. J Neural Transm (Vienna). 2017;124(10):1217–21. https://doi.org/10.1007/s00702-017-1762-3.