



Neurobiology and therapeutic applications of neurotoxins targeting transmitter release



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ABSTRACT

Synaptic transmission is a fundamental neurobiological process enabling exchange of signals between neurons as well as neurons and their non-neuronal effectors. The complex molecular machinery of the synaptic vesicle cycle and transmitter release has emerged and developed in the course of the evolutionary race, to ensure adaptive gain and survival of the fittest. In parallel, a generous arsenal of biomolecules and neuroactive peptides have co-evolved, which selectively target the transmitter release machinery, with the aim of subduing natural rivals or neutralizing prey. With advances in neuropharmacology and quantitative biology, neurotoxins targeting pre-synaptic mechanisms have attracted major interest, revealing considerable potential as carriers of molecular cargo and probes for meddling synaptic transmission mechanisms for research and medical benefit. In this review, we investigate and discuss key facets employed by the most prominent bacterial and animal toxins targeting the presynaptic secretory machinery. We explore the cellular basis and molecular grounds for their tremendous potency and selectivity, with effects on a wide range of neural functions. Finally, we consider the emerging preclinical and clinical data advocating the use of active ingredients of neurotoxins for the advancement of molecular medicine and development of restorative therapies.

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Contents

1. Introduction	136
2. Botulinum toxins	136
3. Tetanus toxin	138
4. Latrotoxin	140
5. Agatoxins	142
6. Conotoxins	143
7. Beta-bungarotoxin	145
8. Dendrotoxins	146
9. Summary and future directions	148
Conflict to interest	150
Acknowledgments	150
References	150

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1. Introduction

The amazing diversity of neuroactive molecules and peptides comprising bio-toxins surpasses that of all man-made synthetic formulas. Over millions of years of evolution and selection using unlimited resources, natural processes have introduced and fine-tuned an incredible wealth of biomolecules and peptides, offering a vast source of raw material for drug discovery. Conceived and refined with the ultimate purpose of assault or defence, biological poisons and venoms have acquired unprecedented potency and selectivity for interference with specific functions, to disrupt or derail a wide range of fundamental neurobiological processes. It is hardly surprising, therefore, that throughout human history, bio-toxins have found a wide range of use, from a wide range of medications to highly sophisticated warfare means (Escoubas & King, 2009; King, 2011; Madsen, 2001; Serna et al., 2018). Recent advances in basic and translational research have illuminated an entirely new dimension for the use of toxins, which allowed the dissection and analysis of essential physiological and neurobiological processes along with the growing recognition of their vast therapeutic potential (Dolly, Lawrence, Meng, Wang, & Ovsepiyan, 2009; Fabbri, Travaglione, Falzano, & Fiorentini, 2008; Ghazaryan et al., 2015; Lewis & Garcia, 2003; Ovsepiyan, O'Leary, Ntziachristos, & Dolly, 2016).

Despite the exhilarating history and variety of applications in the past, the modern era of venom-based drug discovery commenced with the discovery of an inhibitor of angiotensin converting enzyme (ACE) - Captopril, the blockbuster anti-hypertensive compound found in the venom of the viper *Bothrops jararaca* (Creager & Roddy, 1994). Since then, a large-scale hunt for bio-toxin derived peptides has been launched, with numerous ingredients identified, which empower their ample potency, target specificity and high stability, capable of modulating the functions of a wide range of ion channels, receptors and specific sub-cellular processes such as synaptic vesicle trafficking and neurotransmitter release. Given their relatively small size, most toxins can be readily synthesized and modified to accurately fulfil immediate medical needs, thereby offering a virtually unlimited source of biomaterials for developing new classes of bio-therapeutics (Harvey, 2014; King, 2011; Lewis & Garcia, 2003; Utkin, 2015). Advances in high-throughput screening with careful structural and functional characterisation of biomolecules has also accelerated toxin and venom-based drug discovery (Peigneur & Tytgat, 2018; Robinson, Undheim, Ueberheide, & King, 2017). Currently, toxin-derived therapeutics stand alongside numerous approved medications on the market, protected by patents, with their properties increasingly used for the treatment of an extending variety of disorders, including cancer, inflammation, neurological and neurodegenerative disease, cardiovascular ailments and a range of other conditions (Beraud & Chandy, 2011; Hmed, Serria, & Mounir, 2013; Silva et al., 2015). Notwithstanding that only a fraction of bio-therapeutic candidates has been pharmacologically characterized, there is growing recognition of the ever-widening range of their effects on biological processes and mechanisms.

Toxins that have specific neuronal action have become of major interest, owing to their effects on fundamental processes with the capability for targeting/delivering of bio-cargo and therapeutic vectors to neurons and specific neuronal types (Dolly et al., 1994; Lalli, Bohnert, Deinhardt, Verastegui, & Schiavo, 2003; Ovsepiyan, O'Leary, et al., 2016; Singh, 2010). The rapidly expanding usage of botulinum toxins (BoNTs) and their chimeras has played a major role in stimulating interest in the medical utility of bio-toxins, signifying yet another remarkable milestone in the history of medicine, and testifying to the fluidity of the borderlines between poison and remedy, as professed by renowned Paracelsus (Paracelsus, 1538). Indeed, the ability of BoNTs to subdue hyper-active secretory glands and relax tense muscles as well as facilitate targeted delivery of therapeutic cargo or viral vectors to neurons and nerve terminals have taken centre stage in a range of recent studies of bio-toxins under preclinical settings as well as in extensive clinical applications (Dolly, O'Leary, & Lawrence, 2014; Edupuganti et al.,

2012; Jankovic, 2004; O'Leary et al., 2011; O'Leary, Ovsepiyan, Bodeker, & Dolly, 2013; Ovsepiyan, Bodeker, O'Leary, Lawrence, & Oliver, 2015). Such developments, without doubt, stimulated interest and in-depth research culminating in the discovery of an increasing number of molecular targets and potential application areas.

This review aims to present a critique of the most studied neurotoxins targeting transmitter release mechanisms at central and peripheral synapses. Essential facets of presynaptic biology and transmitter release are discussed, with effects of different neurotoxins on various processes critically overviewed from basic and translational neuroscience points of view. Unlike a number of recent specialist reviews (Adams & Berecki, 2013; Dolly et al., 2014; Ovsepiyan et al., 2016; Pirazzini, Rossetto, Eleopra, & Montecucco, 2017; Yan & Wang, 2015) presenting an in-depth coverage of biology and pharmacology of neurotoxins, this study offers a general perspective on prevailing presynaptic neurotoxins, providing a first-hand reference to specialists interested in the biology of neurotoxins as well as neuroscientists and clinicians with a general interest in their translational therapeutic utility.

2. Botulinum toxins

Even though the deadly nature of botulism has been recognized over centuries, the mechanistic grounds for the incredible noxiousness of BoNTs have emerged more recently (Fleck-Derderian et al., 2017; Sobel, 2005; Sobel, 2009), inferring their unique and highly-efficient features (Fig. 1). To date, eight BoNT serotypes (A–G and X) have been identified and characterized, which are produced by gram-positive anaerobic *Clostridium botulinum*, *Clostridium beratii*, *Clostridium butyricum* and *Clostridium argentinensis* as single-chain (SC) proteins (Mr ~150 kDa) (Dolly et al., 2014; Simpson, 2004; Zhang et al., 2017). In addition to these archetypal BoNTs of *Clostridia* origin, recently, two botulinum-like neurotoxins of non-*Clostridia* origin have been identified and characterised (Zhang et al., 2017; Zornetta et al., 2016).

Botulinum toxins are relatively large modular bio-molecules, which upon entry into bodily fluids are converted from an inactive SC into an active di-chain (DC) (known as one of the most poisonous biomaterials) by endogenous proteases. Structurally, BoNTs are very sophisticated and are comprised of a long heavy chain (HC) constituting membrane binding H_C and translocation H_N domains linked to a light chain (LC) protease (Fig. 1A). BoNT HC (~100 kDa) is thought to enable neuronal targeting via high-affinity binding to the neuronal membrane at neuromuscular junctions (NMJ) of motor nerve endings, a step followed by H_N -mediated translocation of the LC (~50 kDa) protease into the neuronal cytosol, causing neuro-paralysis (Brunger & Rummel, 2009; Lacy & Stevens, 1997; Lacy, Tepp, Cohen, DasGupta, & Stevens, 1998; Montal, 2010). The initial interaction of BoNTs with gangliosides and protein-receptors at nerve terminals, known as a binding step, is followed by rapid internalization of the toxin, a process that depends on synaptic activity and recovery of the synaptic vesicle membrane after exocytosis, demonstrated for the first time directly at mammalian motor nerve terminals (Black & Dolly, 1986; Black & Dolly, 1987; Dolly, 2003; Dolly, Black, Williams, & Melling, 1984; Montecucco, 1986). After the uptake, BoNTs are trafficked inside synaptic vesicles, passing through an acidic phase with conformational changes, which facilitate the translocation of the LC metalloprotease into the neuronal cytosol, where it is exposed to the thioredoxin-thioredoxin reductase system, resulting in reduction of the disulfide bridge followed by Hsp90-mediated refolding (Pirazzini et al., 2014; Pirazzini et al., 2016; Pirazzini et al., 2017; Tehran et al., 2017; Tehran & Pirazzini, 2018). The latter step is essential for LC targeting and cleavage of soluble N-ethylmaleimide sensitive factor attachment protein receptor (SNARE) proteins (Fig. 1B, C). Because of the central role of SNAREs in synaptic vesicle docking and their Ca^{2+} -dependent fusion with surface membrane and exocytosis, their cleavage by BoNTs leads to blockade of synaptic transmission (Binz et al., 1990; Blasi et al., 1993; Capogna, Gahwiler, & Thompson, 1996; Foran, Lawrence, Shone, Foster, & Dolly, 1996; Schiavo et al., 1993). Through

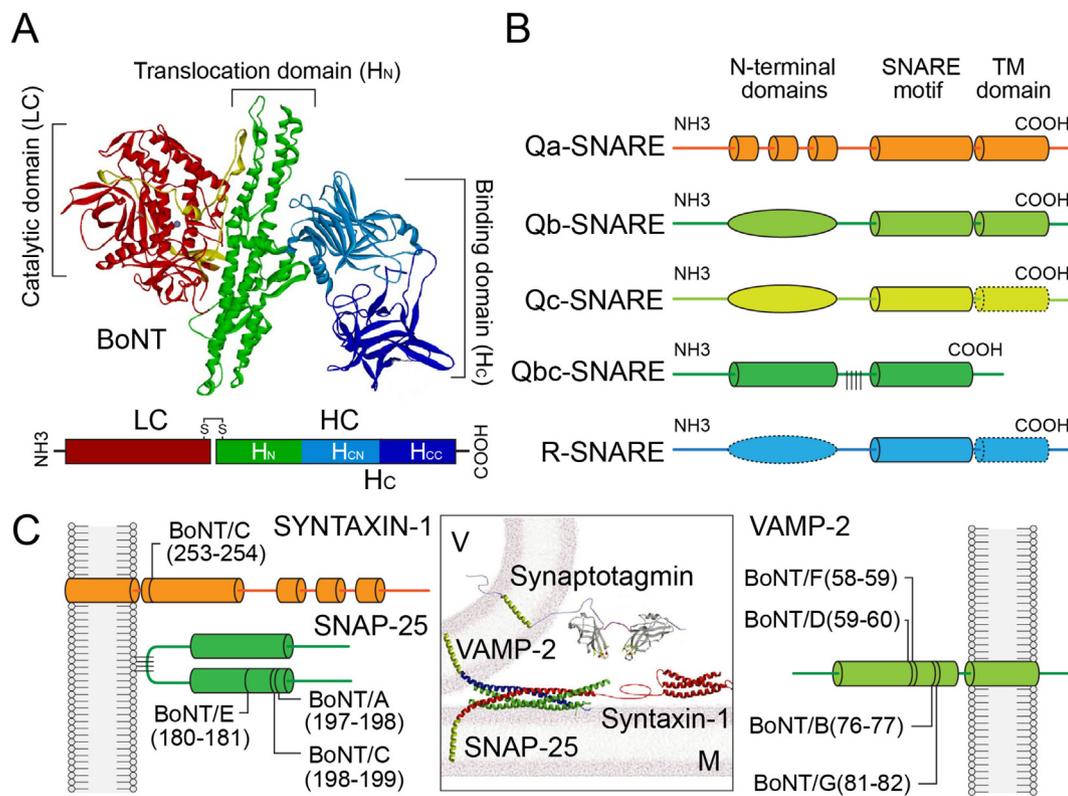


Fig. 1. Botulinum neurotoxins and their synaptic targets. (A) Crystal structure of botulinum neurotoxin type A (BoNT/A) (top) with color coded schematic illustration of various domains (bottom). The C-terminal binding domain (H_{CC}) of the heavy chain (HC) adopts a trefoil fold to bind the membrane surface of neurons while the N-terminal jelly-roll motif (H_{CN}) is proposed to interact with the translocation domain (H_N) to facilitate the delivery of the light chain (LC) protease into the neuronal cytoplasm. The translocation domain is represented by a pair of long helices reminiscent of a viral coiled-coil motif and a long loop (or translocation belt) that wraps around and protects the catalytically active site. The LC contains the conserved HEXXH motif characteristic of zinc-dependent proteases. Adapted with permission from (Ovsepiyan, O'Leary, et al., 2016). (B) Principal SNARE proteins and their schematic structures. The C-terminal of SNAREs corresponds to the transmembrane (TM) domain; the central portion contains the SNARE motif enabling assembly into a super-stable complex that drives membrane fusion, while the N-terminal of Qa-SNAREs (syntaxins in neurons) possesses antiparallel three-helical bundles. In Qbc SNAREs (SNAP-23 and SNAP-25 in neurons), duplicated SNARE motifs are connected by a linker that is frequently palmitoylated (vertical lines) and anchors the protein to the neuronal membrane. During vesicular fusion at nerve terminals Qbc SNAREs contribute two α -helices to the four-helical core complex, with the other two provided by Qa (syntaxins) and R SNAREs (VAMPs). Dashed borders highlight domains that are missing in some subfamily members. Modified with permission from (Jahn & Scheller, 2006). (C) Neuronal SNAREs (left: Syntaxin-1 and SNAP-25; right: VAMP-2) constituting molecular targets of *Clostridial* neurotoxins, which recognize and cleave these proteins at specific scissile bonds (illustration represents rat SNAREs). For further details of SNARE cleavage in various other species, including humans, the readers are referred to (Humeau, Doussau, Grant, & Poulain, 2000). Middle insert: schematic of the surface plasma membrane (M) and vesicle membranes (V) with SNARE complex assembly shown at their interface. A crystal structure of SNARE core complex with four parallel α -helices associated in the ternary complex is adapted from (Sutton, Fasshauer, Jahn, & Brunger, 1998). The C-terminals, which points toward the vesicle and surface membrane are on the left.

such a multistep process, the proteases of BoNT/A and/E reach and cleave the SNARE protein SNAP-25, although at different sites (Figs. 1C, 2C). BoNT/B, /D, /F, /G and /X proteases, on the other hand, cut and neutralize another SNARE protein synaptobrevin, which is known also as vesicle associated protein (VAMP I/II), while the type C1 protease cleaves two SNAREs, SNAP-25 and syntaxin (Figs. 1C, 2C). Recently, two novel botulinum-like non-*Clostridia* neurotoxin BoNT/Wo and BoNT/En have been discovered, with the former targeting VAMP I/II, while the latter cleaves and inactivates VAMP I/II and SNAP-25 proteins (Tehran & Pirazzini, 2018). In this way, after encountering synaptic terminals and delivering their LCs into the neuronal cytosol, BoNTs arrest neurotransmitter release within hours, with inhibitory effects that can last from several days to many months, until the affected nerve terminal resumes exocytosis (Foran, Davletov, & Meunier, 2003; Pirazzini et al., 2017).

To date, the secretion of all transmitter types, from both small and large dense-core vesicles have been shown to be susceptible to the inhibitory effects of BoNTs, provided that the BoNT LC can get into the intracellular environment (Ashton & Dolly, 1988; Ashton & Dolly, 2000; Dolly, 2003; McMahon et al., 1992; Ovsepiyan & Dolly, 2011; Sudhof & Rizo, 2011). From a basic science viewpoint, the variety of BoNT serotypes that cleave one or more SNAREs at different sites is highly advantageous in dissecting the role of individual SNAREs in driving exocytosis.

The usage of such discriminating probes, hence, can yield major insights into the complicated multistage cellular and molecular processes operating at presynaptic terminals. For instance, BoNT/A-induced inhibition of transmitter release from peripheral and central neurons can be reversed by increasing the intracellular Ca^{2+} concentration, while similar manipulations with intracellular Ca^{2+} failed to counter the paralytic effects induced by other serotypes tested so far (Capogna, McKinney, O'Connor, Gahwiler, & Thompson, 1997; Meng et al., 2009; Sakaba, Stein, Jahn, & Neher, 2005). Likewise, the role of different SNAREs in neurotransmitter release at different synapses and neuron types can be dissected using BoNT serotype-specific inhibitors, which showed promise in restoring synapses from paralysis in *ex vivo* and *in vivo* models (Edupuganti et al., 2012; Guo et al., 2015; Kiris, Burnett, Kane, & Bavari, 2014). Importantly, these differences not only have fundamental implications for improving the current understanding of molecular mechanisms that control transmitter release, but, also, for the utility of different BoNTs to target specific mechanisms operating at specialized motor nerve endings in neuromuscular junctions, autonomic neurons and synapses, as well as in pain sensing nociceptors (Dolly et al., 2009).

From a translational viewpoint, elucidation of the multistep processes involving the binding and internalization of BoNTs into synaptic terminals and their action on the secretory machinery, provide a

framework for deciphering their molecular action and key targets for therapeutic interventions. Indeed, BoNTs have been used with astounding success for the treatment of a wide range of neurological conditions related to hyperactivity in the periphery, especially effective in various forms of dystonia and other dysfunctions of involuntary motor movement, including spasticity and glandular hypersecretion (Bentivoglio et al., 2009; Dolly & O'Connell, 2012; Elia, Filippini, Calandrella, & Albanese, 2009; Jankovic, 2004; Jankovic, 2009). Emerging data suggests also the therapeutic utility of BoNTs for the treatment of chronic pain and neuro-inflammatory responses associated with numerous neurological conditions (Aoki, 2003; Oh & Chung, 2015; Qerama, Fuglsang-Frederiksen, & Jensen, 2010). In all of these applications, the therapeutic utility of BoNTs relies primarily upon inhibiting excessive release of classical transmitters and neuromodulator peptides. Importantly, the ameliorative effects are highly specific with readily reversible changes to neural tissue (de Paiva et al., 1993; de Paiva, Meunier, Molgo, Aoki, & Dolly, 1999; Meunier, Schiavo, & Molgo, 2002). The exceptional longevity of BoNT/A action, which appears to be due to the stability of its protease, is also particularly attractive for developing long-acting therapeutics, while shorter acting BoNT/E may afford transient effects lasting over several days (Foran et al., 2003). Thus, in addition to offering a convenient toolkit for advancing fundamental research of synapses and targeting different neuronal types, some BoNTs exhibit promising therapeutic capabilities with potential applications for amelioration of muscle hyperactivity, soothing overactive nociceptors as well as taming overactive secretory glands. Along with natural BoNTs, the toolkit of neurotoxins has become enriched with newly created recombinant BoNT-derived variants and bio-pharmaceuticals, produced by the fusion of different BoNT domains specifically tailored to meet particular needs, or through other molecular (and biochemical) modifications. These include generation of mosaic proteins by fusion of various BoNT moieties (Dolly et al., 2009; Meng et al., 2009; Wang et al., 2008), a number of conjugates of light chain and N-terminus of HC heterodimer (Chaddock et al., 2000; Chaddock & Marks, 2006; Shone, Hambleton, & Melling, 1985) as well as lectin conjugates or other neuron-specific ligands for enhanced targeting and delivery of biomolecules (Chaddock et al., 2000; Chaddock, Purkiss, Duggan, et al., 2000; Duggan et al., 2002). Another approach utilized site-directed mutagenesis to generate non-toxic BoNTs with maintained neuronal binding and delivery of therapeutic payload (O'Leary et al., 2011; O'Leary et al., 2013; Ovsepian et al., 2015; Ovsepian, O'Leary, et al., 2016). Proof of concept for these approaches has already been obtained, affording effective targeting of presynaptic functions and modulating SNARE-dependent synaptic secretion machinery in *in vitro* and *in vivo* models (Edupuganti et al., 2012; O'Leary et al., 2011). Earlier attempts have been also made towards the use of the BoNT H_C binding domain for enhanced delivery of bio-therapeutics to neurons and synapses, with however modest outcome (Bade et al., 2004; Edupuganti et al., 2012; Goodnough et al., 2002; O'Leary et al., 2011), prompting interest in the utility of detoxified full-length botulinum toxin protease mutant (BoTIM), which is superior over its binding H_C domain. Dedicated nano-carriers derived from detoxified full-length BoNTs fused with core streptavidin (CS) have been validated in a range of *in vitro* and *in vivo* pre-clinical applications (Dolly et al., 2014; Edupuganti et al., 2012; O'Leary et al., 2011; Ovsepian, O'Leary, et al., 2016). Taken together, the preclinical and clinical research on BoNTs discussed herein, their validation as nano-carriers for improved neuron targeting and drug delivery have spawned new areas of research and avenues for elucidating further their biology and medical utility, with major translational relevance.

3. Tetanus toxin

As is the case with most potent toxins from bacteria, the tetanus toxin (TeNT) is a highly deadly poison (Fishman, 2009; Montecucco & Schiavo, 1994). It is produced by the anaerobic *Clostridium tetani* germinating primarily in infected wounds. From this location, the toxin is

released via autolysis and systemically spreads into the circulation, reaching peripheral autonomic and motor nerve endings in the NMJ, which represent the primary site of entry into neurons (Cook, Protheroe, & Handel, 2001). While the nature of the TeNT receptor complex at the peripheral synapses has remain elusive over many years, recently it was shown that the presence of nidogens (also known as entactins), which are extracellular matrix linker molecules, are critical for the high-affinity binding and internalization of TeNT (Bercsenyi et al., 2014). Like BoNTs, TeNT is produced as a ~150 kDa single-chain protein, that is post-translationally nicked into a di-chain composed of a ~100 kDa heavy chain (HC) and ~50 kDa light chain (LC) protease linked via a disulfide bridge and non-covalent interactions (Craven & Dawson, 1973; Fishman, 2009; Rossetto, Seveso, Caccin, Schiavo, & Montecucco, 2001). Similar to the blockade of synaptic transmission induced by BoNTs, the inhibition of neurotransmitter release caused by TeNT is a complex and multi-step process. Like BoNTs, TeNT shows excellent neuro-selectivity due to specific receptor-mediated binding to nerve terminals, a characteristic largely attributed to the C-terminal domain of the HC (Fig. 2A). This initial step is followed by internalization of TeNT into nerve terminals. However, unlike BoNTs exerting toxic effects at first entry sites in the periphery, upon internalization, TeNT hijacks the fast intra-axonal transport system of neurons to reach the spinal cord and brain stem (Benecke, Takano, Schmidt, & Henatsch, 1977; Bomba-Warczak et al., 2016; Brooks, Curtis, & Eccles, 1957; Schwab & Thoenen, 1976; Takano, Kirchner, Terhaar, & Tiebert, 1983). Therein, TeNT crosses synapses and enters into the presynaptic terminals of inhibitory interneurons where it blocks the release of neurotransmitters (GABA and glycine), causing disinhibition of large motor neurons that leads to spastic paralysis. The latter, if untreated, causes death, due to violent convulsions driven by muscular hypertonus, and breathing failure (Fishman, 2009). At central synapses, TeNT exerts potent effects at the presynaptic terminals of interneurons, where H_N enables pH-dependent translocation of an active LC protease into the neuronal cytosol (Emsley et al., 2000; Schiavo et al., 1992; Schiavo et al., 1992; Schiavo, Ferrari, Rossetto, & Montecucco, 1991). Upon release from synaptic vesicles, the active protease of TeNT cleaves VAMP I/II, one of three main neuronal SNARE proteins, disabling the formation of the supra-molecular SNARE complex (contributed by VAMP, syntaxin and SNAP-25), a reaction driving synaptic vesicle exocytosis. Thus, despite shared features with BoNTs, there are fundamental differences between the two, responsible for different paralytic effects.

The unique capability of TeNT to target and enter motor nerve terminals and autonomic nerves at the periphery, and travel from there to the central nervous system via retro-axonal transport has generated considerable interest for translational research and clinical application (Ovsepian, O'Leary, et al., 2016). In fact, TeNT is the only known substance that has the potential for selective enhancement of motor functions, capable of overcoming flaccid muscles and muscle weakness caused by brain and spinal cord injuries (Brooks et al., 1957; Dillingham, 2007; Sasse, Conduit, Ryan, Woods, & Tucker, 2005). Observation in humans and experimental animals show that TeNT could be beneficial for inducing a state of elevated activity in a target population of motor neurons and muscles even in vaccinated animals and humans (Fishman, 2009). This unique and highly advantageous feature of TeNT is currently under investigation, with positive attempts reported for its use in treatment of several conditions related to acute and chronic muscle weakness (Fezza et al., 2000; Fishman, 2009). An illustrative example of such application is TeNT injection into the pharyngeal muscle for suppression of snoring and related with it breathing apnoea, which leads to sleep improvement without adverse effects (Sasse et al., 2005). New TeTx therapies that could prevent and reverse muscle atrophy associated with immobility have also been considered, which would complement current rehabilitation regimes for patients with limb trauma (Matthews, Fishman, & Wittenberg, 2014).

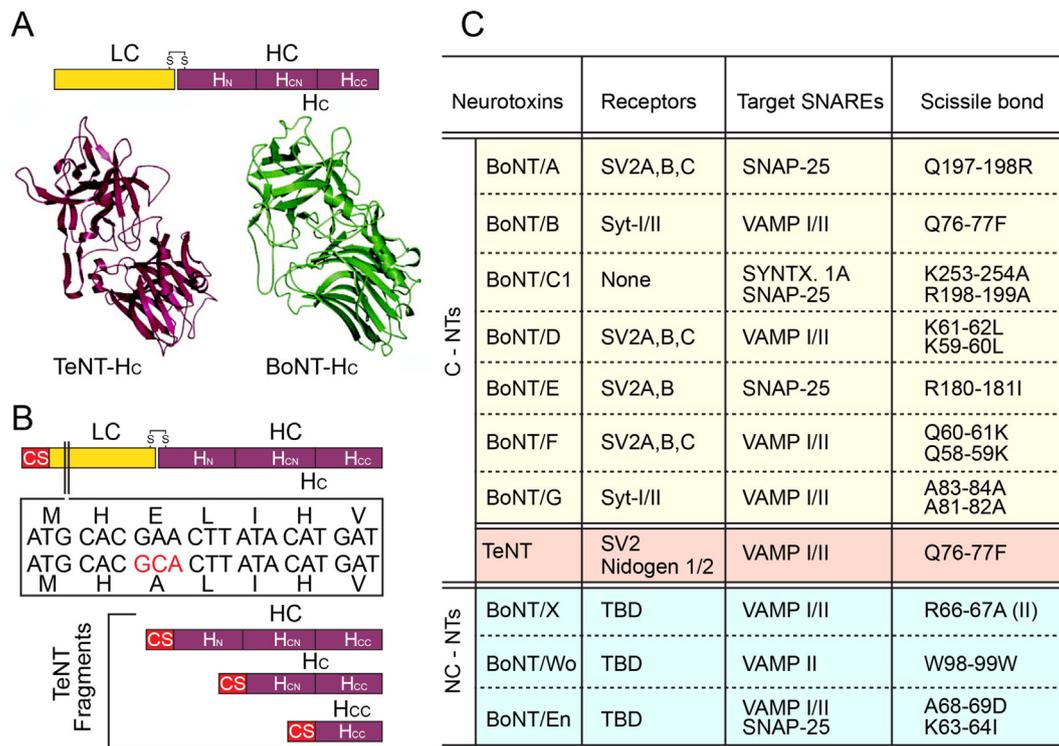


Fig. 2. Tetanus toxin, *Clostridial* and non-*Clostridial* neurotoxins, receptors and SNARE cleavage. (A) Schematized illustration of the structure of TeNT (top) and crystal structure of Hc binding domains of BoNT/A and TeNT (bottom). Note remarkable structural similarities of protein architecture. Adapted, with permission from (Turton, Chaddock, & Acharya, 2002). Similar to BoNTs, the C-terminal binding domain (Hcc) of TeNT adopts a trefoil fold to bind the membrane surface of neurons, while the N-terminal jelly-roll motif interacts with the translocation domain (HN). The light chain (LC) of TeNT, like BoNT LC contains the conserved HEXxH motif characteristic of zinc-dependent proteases. (B) Schematics of TeNT-derived recombinant core streptavidin (CS) fusion proteins: detoxified full-length tetanus toxin (TeTIM) (top) and TeNT binding fragments (TeNT fragments) (bottom) fused with CS made for targeting viral vectors to motor nerve terminals. CS-TeTIM was produced through the fusion of CS and detoxified tetanus toxin (TeNT) (red; E234A point mutation) genes. (C) A summary table of *Clostridial* neurotoxins (the seven serotypes of BoNTs and TeNT) and the recently discovered non-*Clostridial* neurotoxins with their protein receptors, target SNARE proteins and scissile bonds. TBD stands for 'to be defined'.

Another area of TeNT research presenting clinical relevance that has created much interest is its potential as a nano-carrier for neuron targeting and drug/vector delivery to the central nervous system (Ovsepiyan, O'Leary, et al., 2016; Toivonen, Olivan, & Osta, 2010). Given the tight protection of the brain and spinal cord by the blood brain barrier (BBB), which prevents over 98% of small and 100% of large molecules from entry into the central nervous system, the enhanced capacity for cargo and vector delivery by TeNT is highly advantageous for the transfer of therapeutic load or genes to central neurons. Since the first report of the cytoplasmic delivery of TeNT LC by BoNT/A HC in isolated mouse phrenic nerve hemi-diaphragm preparations (Weller, Dauzenroth, Gansel, & Dreyer, 1991), both TeNT and BoNT HC have been utilized to endow neurotropism on different bio-cargo and vectors (Andreu, Fairweather, & Miller, 2008; Bade et al., 2004; Dobrenis, Joseph, & Rattazzi, 1992; Francis et al., 2004; Knight et al., 1999). Some studies however have found that the neuron targeting and trafficking of TeNT is not replicated faithfully by TeNT HC (Fishman, 2009; Fishman & Carrigan, 1987, 1988; Weller et al., 1991; Weller, Taylor, & Habermann, 1986). Moreover, in terms of counteracting the paralytic action of TeNT, a full-length but enzymically inactive mutant of TeNT (TeTIM) is >30 fold more effective than HC (Li et al., 2001), an observation that supports the superiority of TeNT and TeNT toxoid, over its HC in binding to nerve terminals (Fishman, 2009; Weller et al., 1986). The efficacy of the HC translocation or HC-tethered payload delivery into the cytoplasm of the target cell is also controversial and requires further research (Fishman, 2009; Francis et al., 2000).

Hence, while instructive as model carriers and showing promise in preclinical studies *in vitro*, TeNT C-terminal binding fragments have proven to be generally disappointing as delivery vehicles (Fishman, 2009; Ovsepiyan, O'Leary, et al., 2016). This could be due to the close

cooperation between various domains of TeNT for maximal performance, with conclusive data presented recently for better performance of TeTIM over its binding fragments in antagonizing the paralytic effects of TeNT (Li et al., 2001; O'Leary et al., 2013; Ovsepiyan et al., 2015) (Fig. 2B). Indeed, protease inactive mutant BoNT/B (BoTIM/B) or TeTIM fused with core streptavidin have shown superior targeting and delivery capacities as carriers. These new molecules also have been found to be better vehicles for delivery of fluorescence reporters and viral vectors to the motor nerve terminals and spinal cord neurons *ex vivo* as well as *in vivo* (Edupuganti et al., 2012; O'Leary et al., 2011; Ovsepiyan et al., 2015; Ovsepiyan, O'Leary, et al., 2016). In all neuron-binding, internalization and retrograde transport assays, CS-TeTIM out-performed CS fusion proteins of HC or Hc, with CS of CS-TeTIM readily detectable in axon terminals of motor neurons and in spinal cord neurons (O'Leary et al., 2013; Ovsepiyan et al., 2015; Ovsepiyan, O'Leary, et al., 2016). These findings agree with data showing that detoxified TeNT is superior to TeNT binding domains at counteracting TeNT-induced cleavage of VAMP I/II in rat cultured neurons (Ovsepiyan et al., 2015). Results from studies of chimeric BoNTs and TeNT further support the notion of cooperation between different domains, warranting their superior performance and potency (Ovsepiyan et al., 2015; Ovsepiyan, O'Leary, et al., 2016; Wang et al., 2008; Wang et al., 2012).

All in all, TeNT emerges to have broadly two major areas for therapeutic application. Firstly, the unique ability for selective enhancement of motor functions creates the potential for overcoming flaccid muscles and muscle weakness caused by brain and spinal cord injuries, as well as for treatment of other conditions related with muscle weakness. Secondly, its excellent capacity to target nerve endings and penetrate from the periphery to central neurons makes is highly valuable as a potential nano-carrier for cargo and therapeutic gene delivery to central

neurons. Despite major structural similarities with BoNTs, TeNT displays several important differences, that make this amazing molecule highly interesting in its own right (Fig. 2A, C). Careful comparison of multistep reactions involving BoNTs, TeNT and their proteolytic targets is warranted for further advancement of carriers and bio-therapeutics, exhibiting uncompromised performance. While concerns remain over risks related to residual toxicity of TeNT-derived carriers, advances in recombinant technologies and selective immuno-suppressants inspire optimism in the future medical usage of these wonder molecules. Indeed, meeting many important criteria, TeTIM holds considerable potential for use as a carrier in a variety of neurological and degenerative diseases affecting motor neurons, including ALS and primary lateral sclerosis, various forms of neuromuscular atrophy and palsy.

4. Latrotoxin

α -Latrotoxin is another potent toxin targeting the molecular machinery of exocytosis, which has a high affinity for receptors that are expressed specifically on neuronal and endocrine cells. α -Latrotoxin is enriched in the venom of the widow spiders of the genus *Latrodectus*, with the most widely known amongst them the black widow, *L. mactans*. In addition to α -latrotoxin, the venom of *Latrodectus* contains a plethora of other peptides displaying a range of activities (Yan & Wang, 2015). Most of these peptides target neuromuscular transmission in insects (α - and δ -latroinsectotoxins) or crustacea (α -latrocrustotoxin) (Duan et al., 2006), which constitute the natural prey of the black widow spider. From the rich cocktail of toxins produced by this spider, only α -latrotoxin is specific for vertebrates (Garb & Hayashi, 2013). Despite the scaremongering, bites from a black widow spider do not present a major health problem for humans because they rarely cause serious disease (Ryan, Buckley, & Graudins, 2017; Sudhof, 2001). Only in the most serious cases do black widow spider bites cause latrodectism, a syndrome consisting of generalized muscle pain, abdominal cramps, profuse sweating, a rise in blood pressure and tachycardia, with death encountered only on very rare occasions (Sudhof, 2001; White & Weinstein, 2015). The distress caused by α -latrotoxin is thought to be due to dramatically increased release of neurotransmitters. When injected systemically, the primary target of α -latrotoxin is the neuromuscular junction, where the toxin triggers exocytosis of acetylcholine contained in clear synaptic vesicles (Ceccarelli, Hurlbut, & Iezzi, 1988; Matteoli et al., 1988). Induced by α -latrotoxin, secretion of peptides and catecholamines from sensory neurons and endocrine cells has also been widely documented (Barnett, Liu, & Misler, 1996; De Potter, Partoens, Schoups, Llona, & Coen, 1997; Meldolesi, Madeddu, Torda, Gatti, & Niuatta, 1983). In fact, α -latrotoxin has proved capable of activating massive release of all mediators from all secretory cell types tested so far (Silva, Lelianaova, Hopkins, Volynski, & Ushkaryov, 2009).

Due to potent synaptic effects, α -latrotoxin is a useful molecular probe for studying neurotransmission in mammals and humans. Fig. 3 (A–D) presents an overview of its structure and receptors along with the mechanisms of the effects of α -latrotoxin on synaptic transmission in vertebrates. Similar to other toxins targeting the presynaptic release machinery, the action of α -latrotoxin on synaptic release is a complex and multi-step process. To induce synaptic activity changes, the toxin should initially bind to specific receptors on synaptic terminals (Sudhof, 2001). Analysis of α -latrotoxin effects revealed that it stimulates transmitter release by two distinct mechanisms, both reliant upon toxin binding to three structurally unrelated receptors: neurexins, latrophilin 1 and receptor-like protein tyrosine phosphatase σ (McMahon, Rosenthal, Meldolesi, & Nicholls, 1990; Rosenthal, Zacchetti, Madeddu, & Meldolesi, 1990; Ushkaryov, Rohou, & Sugita, 2008) (Fig. 3B, C). Neurexins are brain-specific cell adhesion molecules, which bind to α -latrotoxin in a Ca^{2+} -dependent manner. After binding, α -latrotoxin exerts its effects via two pathways: (1) Ca^{2+} -dependent, which involves α -latrotoxin insertion into the plasma membrane and

pore formation (Orlova et al., 2000) and (2) Ca^{2+} independent action, reliant on receptor-mediated signalling (Ashton et al., 2001; Davletov et al., 1998). In the presence of extracellular Ca^{2+} , thus, α -latrotoxin bound to latrophilin operates as a Ca^{2+} ionophore while in the absence of Ca^{2+} , the effects of α -latrotoxin are due to stimulation of signal transduction and enhancement of synaptic activity by phosphorylation of SNARE proteins such as SNAP-23, syntaxin IV, and VAMP VIII, through protein kinase C (PKC) as well as PKC-independent pathways (Hiramatsu, Tadokoro, Nakanishi, & Hirashima, 2010; Yan & Wang, 2015). The latter mechanisms rely on latrophilin binding to G proteins, namely $\text{G}\alpha/\text{o}$ and $\text{G}\alpha\text{q}/11$ (Rahman et al., 1999; Serova et al., 2008), which leads to activation of phospholipase C (PLC) and efflux of Ca^{2+} from internal stores with elevation of cytoplasmic Ca^{2+} , which subsequently triggers transmitter release (Fig. 3D).

It must be stressed that the effects of α -latrotoxin on neurotransmitter release in the absence or presence of Ca^{2+} are fundamentally different and show distinct kinetic and dynamic characteristics (Silva, Suckling, & Ushkaryov, 2009; Tsang, Elrick, & Charlton, 2000). In general, most of the toxins effects are induced by elevated Ca^{2+} influx via presynaptic channel-pores (Davletov, Shamotienko, Lelianaova, Grishin, & Ushkaryov, 1996), requiring the involvement of VAMP I/II and SNAP-25 (Ashton et al., 2001; Capogna et al., 1996; Davletov et al., 1996; Davletov et al., 1998). Through a combination of genetic methods and electrophysiology, it has been shown that α -latrotoxin induced Ca^{2+} -dependent transmitter release recruits VAMP plus SNAP-25 and active zone protein Munc13-1 (Deak et al., 2009). In contrast, Ca^{2+} -independent release uses a non-classical pathway, which does not involve the regulated exocytosis machinery and departs from the canonical action-potential induced secretory pathway (Capogna et al., 1996; Deak et al., 2009; Ushkaryov et al., 2008). The latter ability makes α -latrotoxin uniquely potent and a ubiquitous activator of secretion, capable of depleting even BoNT/A-treated presynaptic terminals as well as stimulating transmission at synapses lacking one or another SNAREs (Deak et al., 2009; Mesngon & McNutt, 2011). Of note, at low amounts (pM), the neuro-stimulatory effects of α -latrotoxin causes no detectable morphological changes, while at high dose (nM), the massive synaptic activity induced can be followed by morphological changes in nerve terminals and degeneration of nerve terminals (Linial, Ilouz, & Feinstein, 1995; Yan & Wang, 2015).

Because of the potent and multifaceted effects on transmitter release, α -latrotoxin has been used as a probe for studies of fundamental molecular and cellular processes driving and modulating exocytosis (Sudhof, 2001; Sudhof & Rizo, 2011; Ushkaryov et al., 2008). Even though the actions of this toxin in various systems are well-documented, numerous questions remain concerning the underlying molecular processes. Indeed, after all the years of α -latrotoxin use in research, the understanding of its Ca^{2+} -independent effects remain incomplete (Silva, Suckling, & Ushkaryov, 2009). It is expected, therefore, that novel recombinant variants of toxin and more unconventional approaches will have to be employed to address remaining questions. In particular, it is of major interest to work out what is the physiological role of α -latrotoxin receptors and what are the functional consequences of their interactions with endogenous ligands? Likewise, the signalling mechanisms and molecular pathways mediating α -latrotoxin effects are a matter of great interest due to the unique ability of the toxin to facilitate transmitter release at central and peripheral synapses.

The potency and selectivity of targeting synaptic mechanisms endow α -latrotoxin with unique therapeutic potentials, which include countering the paralytic effects of BoNTs or overcoming long-lasting inhibition, neuromuscular paralysis and synaptic weakness (Duregotti et al., 2015). Research in this direction has already shown that both, the severity and duration of paralysis induced by BoNTs could be lessened by α -latrotoxin (Mesngon & McNutt, 2011) leading to complete regeneration of the NMJ (Duregotti et al., 2015). In light of the poor efficacy of currently tested BoNT inhibitors, this finding stimulated interest in α -latrotoxin as a potential lead for developing a means to

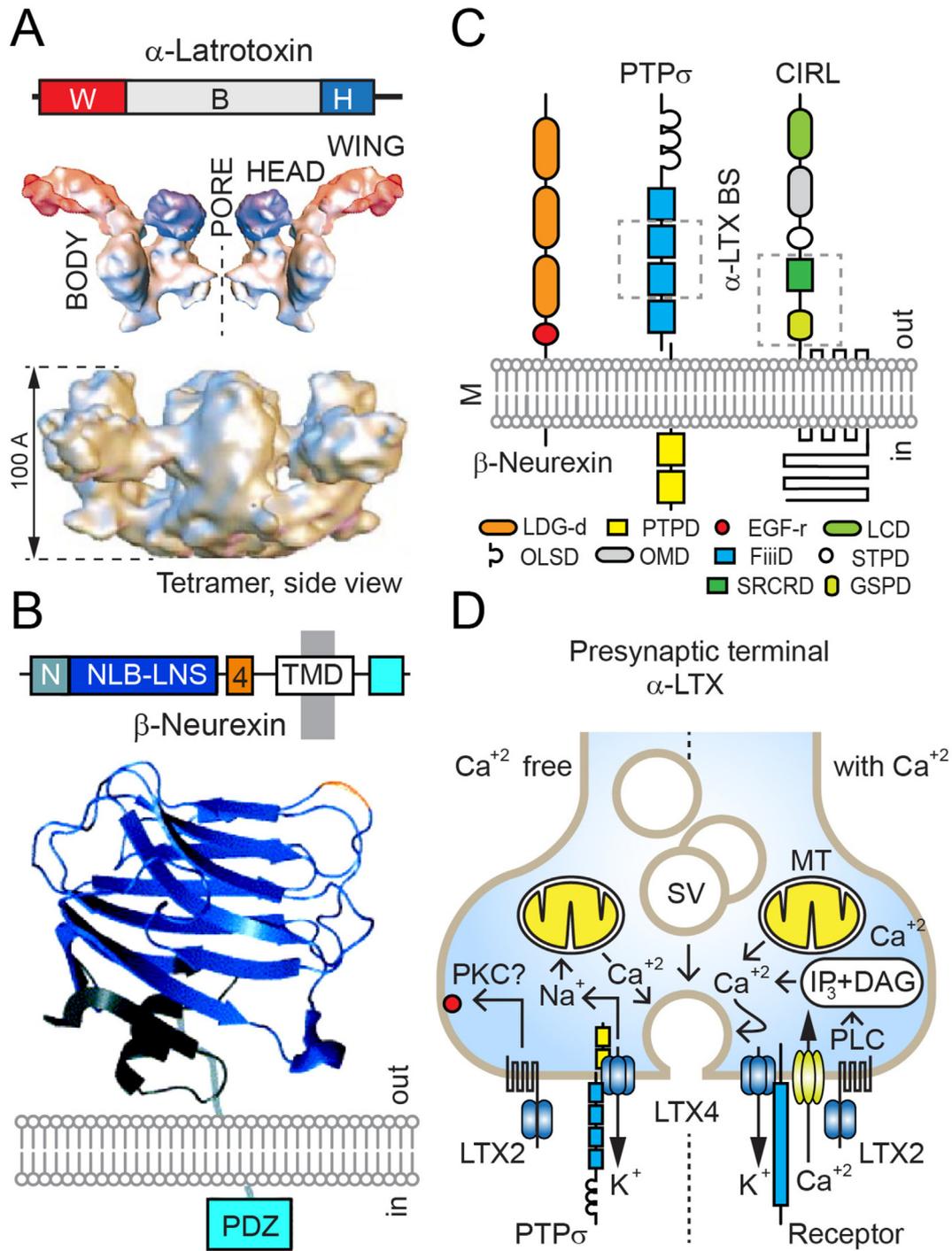


Fig. 3. Schematics of Latrotoxin structure, its receptors and action mechanisms at the presynaptic terminal. (A) Top: schematized α -latrotoxin (LTX) with three structural domains coded in different colors: wing – red (W); body – grey (B) and head– blue (head). The wing domain is the putative receptor-binding domain; the body makes up the transmembrane domain while the head makes the channel mouth. Middle and bottom panels – a side view of α -LTX dimer with its structural subdomains and tetramer, respectively. Through assembly into tetramers, α -LTX forms an aqueous pore mediating Ca^{2+} influx. Crystal structure panels are adapted with permission from (Orlova et al., 2000). (B) Schematic diagram (top) of the structure of the α -LTX receptor β -Neurexin and with corresponding 3D reconstruction (bottom). β -Neurexins are composed of an extracellular N-terminal sequence (N) that is specific to β -neurexins, a single LNS (laminin, neurexin, sex-hormone binding globulin) domain that is essential for neuroligin binding (NLB) (blue yonder color) followed by Band4.1 actin binding domain (orange color) and a transmembrane domain (TMD) (white color) linked to a cytoplasmic tail that contains a PDZ-interaction site on the C-terminus (light blue color). Note that the putative monomer of β -neurexin-1 contains two seven-stranded β -sheets that form a fold, and splice sites that are localized within loops at the edge of the fold (orange color indicated by arrow), which might act as a protein-interaction surface. *Neuroligins* preferentially *bind to β -neurexins* without an *insert*. It is attached to the surface membrane through TMD and is stabilized by PDZ. Adapted with permission from (Dean & Dresbach, 2006). (C) A schematic of the domain structure of all three α -LTX receptors: M – membrane; LDG-d – Laminin A G-domain; EGF-r – EGF repeat; OLS – O-linked sugar domain; FiiiD – Fibronectin III domain; PTP – Protein tyrosine phosphatase; PTPD – PTP domain; LCD – Lectin domain; OMD – olfactomedin domain; STPD – STP-rich domain; SRCRD – Secretin receptor cys-rich domain; GSPD – GSP domain. Schematics are adapted with modifications from (Krasnoperov et al., 2002). (D) Diverse mechanisms of α -LTX action at the neurosecretory machinery. Right, in the presence of extracellular Ca^{2+} and left in the absence of Ca^{2+} ; the pathways shown are briefly described in the text. For more information, the readers are referred to (Ushkaryov et al., 2008). DAG – diacyl-glycerol; LTX2 – α -LTX dimer; LTX4 – α -LTX tetramer; MT – mitochondria; PLC – phospholipase C; IP_3 – inositol 3 phosphate. Possible pathways for Ca^{2+} -independent exocytosis include: (1) high-concentration of Na^+ mimicking Ca^{2+} and the internalized domains of α -LTX interacting with components of the exocytosis machinery and triggering exocytosis.

counter synaptic weakness caused by BoNTs (Mesngon & McNutt, 2011), independently or in combination with currently available small molecular BoNT inhibitors. It is important to note that both, functional and structural effects of α -latrotoxin at motor nerve terminals are completely reversible, hence, providing a useful model for studying synaptic regeneration after neuromuscular paralysis (Duregotti et al., 2015; Rigoni & Montecucco, 2017). Another area of potential interest for α -latrotoxin is its utility for activation of secretion independently from membrane voltage and Ca^{2+} influx. As a potent inducer of Ca^{2+} -independent exocytosis, α -latrotoxin holds promise for ameliorating or correcting dysfunction and disease related to reduced release of transmitters and hormones targeting nervous and cardiovascular functions as well as metabolic processes and disease, including type I diabetes (Holz & Habener, 1998; Saez et al., 2010). Finally, the structural homology of α -latrotoxin to glucagon-like peptide -1 (GLP1) has been suggested to present opportunities for its use as a regulator of food intake, applicable to the treatment of obesity and diabetes and related chronic metabolic disorders (Gejl et al., 2017; Lewis & Garcia, 2003; Perry & Greig, 2003).

5. Agatoxins

Unlike neurotoxins that directly target the molecular machinery of exocytosis, there are several families of neurotoxins which interfere with transmitter release indirectly. Amongst these, peptides modulating presynaptic ion channels have generated much interest due to their remarkable potency and relevance to drug-development across several fields (Harvey, 2014; Kaczorowski, McManus, Priest, & Garcia, 2008; Terlau & Olivera, 2004). Toxins targeting Ca^{2+} channels are of special interest due to the central role played by voltage-gated Ca^{2+} currents in triggering transmitter release at central and peripheral synapses. Much effort has been made in investigating the pharmacology of bio-toxins that modify properties of voltage gated Ca^{2+} channels (Ca_v), with agatoxins from the funnel web spider *Agelenopsis aperta* venom holding the centre stage. Currently, three structural subclasses, α -, μ - and ω -agatoxins, are distinguished, which are selective for ligand-gated cation channels, Na_v and Ca_v channels, respectively (Adams, 2004; Nimrich & Gross, 2012; Pringos, Vignes, Martinez, & Rolland, 2011). Over 33 types of α -agatoxins have been identified, which are composed of polyamines attached to the aromatic moiety antagonizing NMDA and AMPA receptors (Bixel et al., 2001; Parks, Artman, Alasti, & Nemeth, 1991; Usherwood & Blagbrough, 1991). These toxins target both the pre- and post-synaptic compartments of the synapse, with their effects documented in insects and mammals (Parks et al., 1991; Quistad et al., 1990). μ -Agatoxins, on the other hand have 6 subtypes, composed of 35–37 amino acid C-terminally amidated peptides constrained by four disulfide-bonds (Skinner et al., 1989). These toxins are specialized for insects, leading to increased voltage-sensitivity of axons and promoting transmitter release with repetitive firing of motor neurons, causing paralysis (Prikhodko et al., 1996). Finally, ω -agatoxins target voltage-gated (post-) and pre-synaptic Ca_v , affording selective probes for analyzing regulated synaptic transmission in the central nervous system and at the periphery (Fig. 4A–C).

Produced as a single polypeptide chain precursor, ω -agatoxins are post-translationally processed with removal of a hepta-peptide and conversion of L- to D-serine, leading to formation of a mature di-chain. Based on structural differences and selectivity for Ca_v channel subunits, four different groups of ω -agatoxins have been distinguished (Fig. 4A), which modify Ca^{2+} currents either via changing channel gating properties or by blockade of the channel pore (Bourinet & Zamponi, 2017). Accordingly, type I, II and IV ω -agatoxins are known to block Ca^{2+} currents mediated via L, N and P type voltage-gated Ca^{2+} channels, respectively, while type III ω -agatoxin non-selectively blocks currents mediated by all neuronal high-voltage gated Ca^{2+} channels. Each ω -agatoxin blocks synaptic transmission only partly if applied

alone, but they virtually abolish synaptic release if working together in an additive mode (Pringos et al., 2011). Due to drastic and selective action on the neuronal system of insects, some ω -agatoxins have been evaluated as candidate bio-pesticides (Prikhodko et al., 1996; Pringos et al., 2011). ω -Agatoxin groups can be divided onto sub-groups with ω -agatoxins-IIIa and ω -agatoxins-IVa targeting most potently vertebrate Ca_v channels, while ω -agatoxins-IIIa display selectivity for high-voltage-activated L-, N-, P/Q, and R-type channels (Cohen et al., 1992; Mintz et al., 1992; Yan & Adams, 2000). ω -Agatoxin-IVa, on the other hand, is a potent and selective blocker of P-type Ca_v currents, sparing other Ca_v channel subunits (McDonough, Boland, Mintz, & Bean, 2002) (Fig. 4A). Even through ω -agatoxin IVa and ω -agatoxin IVb share conserved cysteine residues (Fig. 4B, C) (Ryu et al., 2017), the high-selectivity of ω -agatoxin IVa for Ca^{2+} currents mediated via the P-type channel has made it a valuable tool for pharmacological isolation and analysis of P-type Ca^{2+} currents in a wide range of neuronal preparations (Llinas, Sugimori, Hillman, & Cherksey, 1992; Randall & Tsien, 1997). Capitalizing on this unique feature of ω -agatoxin IVa and selective enrichment of the P-type channel in specific neuronal compartments of different neuron types as well as brain structures, a range of neurophysiological discoveries on mechanisms of neurotransmitter release and postsynaptic excitability have been made (Llinas, 2014; Llinas et al., 1992; Ovsepian & Friel, 2008; Uchitel et al., 1992). Unlike most spider toxins-derived Ca^{2+} channel blockers obstructing the ion channel pore, ω -agatoxin IVa appears to block the P-type current by binding close to the external mouth of the channel linked to gating, shifting the activation voltage of the channel towards more positive potentials and stabilizing it in a closed state (McDonough et al., 2002; Mintz et al., 1992). Interestingly, although ω -agatoxin IVa blocks neurotransmission at neuromuscular junctions of the rat, crab, and crayfish, it has no effect in equivalent synapses of insects (Meir et al., 1999).

While the potency and selectivity of ω -agatoxin IIIa and ω -agatoxin IVa for high-voltage activated Ca^{2+} channels hold major promise for manipulations of neural excitability and synaptic functions in selected groups of neurons, further research is necessary for an all-inclusive understanding of their pharmacological profile and specificity, in order to clearly delineate areas for their therapeutic usage (Inagaki, Frank, Usachev, Benveniste, & Lee, 2014; Pringos et al., 2011; Saez et al., 2010). Research in this direction has been facilitated by recent studies revealing the atomic resolution structure of voltage-gated Ca^{2+} channels, which elucidate fundamental facets of their biology and pharmacology (Fig. 4D). The ubiquitous prevalence and key role of high-voltage gated Ca^{2+} channels in triggering synaptic transmission throughout the central and peripheral nervous system make this family of ion channels especially interesting and medically relevant. Evidence from behavioural and electrophysiological reports showed the remarkable modulation of spinal nociceptive processing by ω -agatoxin IVa sensitive P-type channels (Diaz & Dickenson, 1997; Park & Luo, 2010), with its intrathecal administration decreasing the late phase of nociceptive behaviour in a formalin-induced animal model (Malmberg & Yaksh, 1994). ω -Agatoxin IVa also proved beneficial in preventing the development of secondary hyperalgesia and allodynia after an intradermal injection of capsaicin (Sluka, 1997) or progression of inflammation in the knee joint (Sluka, 1998) when applied via micro-dialysis, through a fibre implanted in the spinal dorsal horn. This finding suggests the possible use of ω -agatoxin IVa for the alleviation of inflammation-related sensitization of neural circuits. In recordings from spinal cord sensory interneurons, ω -agatoxin IVa reduced discharges of nociceptive neurons in the late phase of the formalin response (Diaz & Dickenson, 1997). In the knee joint proprioceptor neurons of the spinal cord, ω -agatoxin IVa also decreased the neuronal response to innocuous and noxious pressure, in carrageenan induced inflammation models (Nebe, Vanegas, Neugebauer, & Schaible, 1997). The same group subsequently demonstrated that P-type channels may contribute to inflammation-related enhanced excitability of spinal cord neurons, due to excessive release of modulator peptides and transmitters at

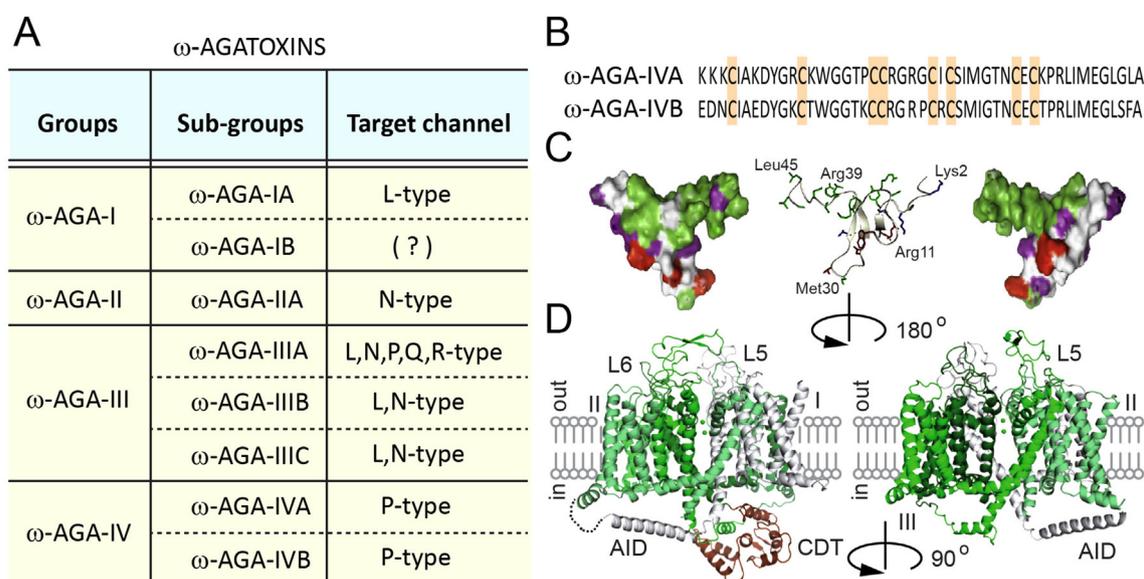


Fig. 4. Ω -Agatoxins and presynaptic voltage-gated Ca^{2+} channels. (A) A summary table listing four groups of ω -agatoxins, with their sub-groups and target Ca^{2+} channel types. (B) Sequence alignment of ω -Aga-IVA and ω -Aga-IVB, which show the most promising characteristics as therapeutic candidates, with conserved cysteine residues highlighted in orange color. Adapted with permission from (Adams, 2004). (C) 3D structure of ω -Aga-IVA in micelles (left and right) and the ribbon structure with the lowest-energy (middle). Color coded models for residues with significant chemical shift perturbations (CSPs): red, 0.05 parts per million (ppm) < d < 0.01 ppm, purple, 0.1 ppm < d < 0.4 ppm and green, d > 0.4 ppm. Modified and adapted with permission from (Ryu et al., 2017). (D) The crystal structure of the α -subunit of L-type ($\text{Ca}_v1.1$) channel (side view, two perspectives with 90° rotation) embedded in the lipid bilayer of the surface membrane. The tentatively assigned Ca^{2+} ions in the selectivity filter vestibule are presented within the ion channel pore as green spheres. The structure shown here was primarily modelled and refined with a 3.6Å class I electron microscopic map. AID – $\alpha 1$ interaction domain and CTD – C terminal domain, respectively.

Modified and adapted with permission from (Wu et al., 2015).

central primary sensory inputs, with activation of corresponding receptors. Taken together, these findings imply that ω -agatoxin sensitive P/Q-, R- and N-type channels can take part in inflammation related central sensitization, and present a therapeutic candidate for treatment of enhanced nociception and for general anti-nociceptive therapy (Lewis, Dutertre, Vetter, & Christie, 2012; Yaksh, 2006). The medical utility of selective P-type channel blockers has also been discussed in the context of treatment of Ca_v channelopathies. Through attenuation of the hyper-excitability of cortical circuits, ω -agatoxin is expected to ameliorate the fits of episodic ataxia, seizures and migraine headaches. By lowering cortical excitability, the P/Q-type Ca^{2+} channel blockade will attenuate synaptic transmission and can decrease the activity in cortical circuits. Several studies have shown that a selective P-type channel blockade with ω -agatoxin IVA can prevent cortical spreading depression in migraine models (Kunkler & Kraig, 2004; Tottene, Urbani, & Pietrobon, 2011), an advantageous feature stimulating further research to harvest the beneficial potentials of this neurotoxin family.

Overall, the results of the studies discussed above encourage further research and development of new approaches, to take advantage of selective Ca^{2+} channel blocking agatoxins and their synthetic analogs, with their preclinical application for treatment of a range of conditions, including enhanced nociception and central sensitization, pathological activity of brain circuits and prophylactic treatment of channel hyperactivity. Better understanding of the biology of agatoxins and their effects on ion channels should facilitate clinical translation and the design of new classes of medication for better control of presynaptic Ca^{2+} channel functions.

6. Conotoxins

The remarkable pharmacological diversity of toxins produced by marine cone snails that are endowed with high selectivity and potency has been well recognized and has become of a major interest for therapeutic screening. The group of cone snails (genus *Conus*) is comprised of over 800 predatory species (Jimenez-Tenorio & Tucker, 2009) with each containing 50–200 distinct biologically-active peptides empowering

their highly evolved hunting strategies (Jenkins & Van Houtan, 2016). The venom gland of each cone species can secrete large amounts of unique neurotoxic peptides, commonly referred to as conopeptides, with most of them exhibiting a broad range of pharmacological activities. More than 80,000 natural toxins exist in various cone snails around the world, which render them one of the largest libraries of natural candidates for drug development (Daly & Craik, 2009; Livett, Gayler, & Khalil, 2004; Olivera, Seger, Horvath, & Fedosov, 2015; Vetter & Lewis, 2012). As it emerges, in the process of venom production, cone snails utilize a strategy equivalent to a ‘peptide combinatorial-library’ system, which facilitates the generation of a wide diversity of novel pharmacologically-active components through a hyper-mutation process (Olivera et al., 1985). The resultant rich collection of conopeptides is highly advantageous for meeting the biological needs of cone snails in a turbulent environment of fast-moving prey, presenting a major survival challenge, which cone snails have overcome by advancing their sophisticated venomous apparatus for the synthesis, storage and delivery of an impressive diversity of toxins (Olivera et al., 2015).

Based on molecular structure and cysteine composition, conopeptides are divided into two main groups: (1) conopeptides that lack or have only one disulfide cross-link and (2) disulfide-rich conopeptides, or active toxic peptides, which represent predominant venom constituents. Fig. 5(A, B) presents a summary table of seven super-families of S-S rich conopeptides, with their families and target channels/ligand-gated receptors with schematic representation of their expression sites in a primary sensory neuron. Typical conotoxins are small (12–30 amino acids) and exhibit highly constrained structures stabilized by intramolecular bridges fine-tuned by post-translational modifications (Fig. 5C, D). The vast diversity of conotoxins mirrors the multiplicity of their targets. Although conotoxins are remarkably miscellaneous in terms of their structure and function, in general, they fall into several distinct categories, based on sequence homology, cysteine bond structure, and functions, with most families described as targeting receptors and ion channels associated with the nervous system or muscle activity. Each superfamily has a remarkable structural (distinct cysteine arrangements) and functional diversity, with α -, μ - and

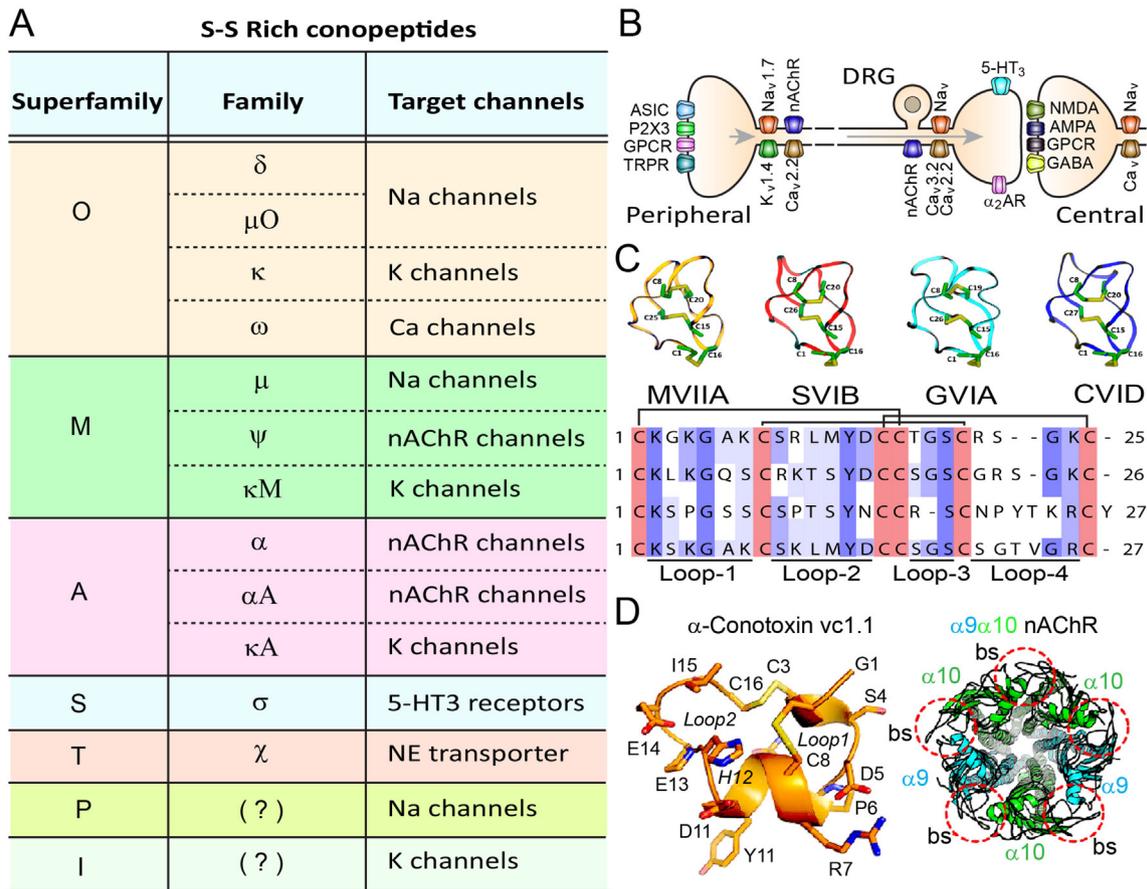


Fig. 5. Pharmacological versatility and molecular targets of conotoxins in central and sensory neurons. (A) A summary table of seven super-families of S-S rich conopeptides, with their families and target channels/ligand-gated receptors in neurons. (B) Schematic diagram of the molecular targets of conotoxins and their location at peripheral nerve endings of primary sensory neurons and central synapses implicated in conotoxin-derived anti-nociceptive therapies. ASIC – acid sensitive ion channel; P2X₃ – P2X purinoceptor 3; GPCR – G-protein coupled receptor; TRPR – transient receptor potential receptor; nAChR – nicotinic acetylcholine receptor; K_v – voltage-gated potassium channel; Nav – voltage-gated sodium channel; Ca_v – voltage-gated calcium channel; 5-HT – serotonin receptor; GABA – gamma amino-butyric acid receptor; AMPA and NMDA – α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and N-methyl-D- aspartate glutamatergic receptors. (C) The backbones of ω -conotoxins are presented as a ribbon diagram (upper). Structural differences between various ω -conotoxins (listed above) with a multiple sequence alignment of four of the most prevalent ω -conotoxins colored by their degree of conservation: from white (not conserved) to dark blue (highly conserved) (top and bottom, respectively). Structural alignment of the 3D rendered structures of MVIIA, SVIB, GVIA and CVID represent differences in their spatial conformation. Adapted and modified with permission from (Ramirez, Gonzalez, Fissore, & Carvacho, 2017). (D) The structure of Vc1.1 solved using NMR-derived distance and angle restraints, with the backbone and side chains shown (left). Structure of nAChR (aerial view with toxin) with conotoxin-binding sites corresponding to each subunit marked in red circles.

Adapted with permission from (Yu, Kompella, Adams, Craik, & Kaas, 2013).

ω -conotoxins representing the best characterized families. The presynaptic effects of conotoxins on neurotransmission are largely due to inhibition of voltage-gated Ca²⁺ channels. From 14 ω -conotoxin variants characterized, ω -conotoxin-GVIA from *Conus geographus* and ω -conotoxin MVIIA from *Conus magus* exhibit high affinity and selectivity for blocking N-type Ca²⁺ channels (Ca_v2.2), while ω -conotoxins-MVIIA reversibly block P/Q-type (Ca_v2.1) channels (Bourinet & Zamponi, 2017). Unlike ω -agatoxins utilizing several mechanisms for blockade of the voltage gating of Ca²⁺ channels, all ω -conotoxins prevent Ca²⁺ influx by occluding the pore through tight binding and generally slow dissociation (Boland, Morrill, & Bean, 1994; Ellinor, Zhang, Horne, & Tsien, 1994; Mintz et al., 1992). Similar mechanisms also mediate the α -conotoxin-induced blockade of the nicotinic receptor-channel complex (Fig. 5D). The potent and ubiquitous capability of conotoxins as Ca²⁺ channels blockers has been widely employed for basic neuroscience research for the analysis of neuronal voltage-gated Ca²⁺ currents, as well as for therapeutic intervention with the neurotransmission machinery. The latter makes conotoxins especially attractive for pharmacological use as a source for new drug discovery (Livett et al., 2004; Twede, Miljanich, Olivera, & Bulaj, 2009). The medical use of ω -conotoxins is largely viewed in conjunction with its potent and specific targeting of presynaptic Ca²⁺ currents in both the

central and peripheral nervous system. Potential areas of interest include but are not limited to epilepsy, neuromuscular disorders and other neurological diseases associated with dysregulation of synaptic activity. Importantly, ω -conotoxins have displayed a highly beneficial characteristic as blockers of voltage-gated Ca²⁺ channels of nociceptors, showing analgesic effects. In this regard, a synthetic form of ω -conotoxin MVIIA (ziconotide, also known as Prialt) has generated major interest for being the first ω -conotoxin to enter into clinical trials, and was approved by the FDA for the management of chronic pain as well as cancer- or AIDS-related neuropathy (Patel, Montagut-Bordas, & Dickenson, 2017). Isolated from *C. magus*, ω -conotoxin MVIIA inhibits neurotransmitter release through blockade of N-type Ca²⁺ channels and attenuates nociception in a variety of animal models, including in a persistent pain model (i.e. formalin test), post-operative pain, chronic inflammatory pain and neuropathic pain (Layer & McIntosh, 2006; Lee et al., 2010). Remarkably, ω -conotoxin MVIIA is effective in morphine tolerant rats with its prolonged intrathecal infusion not producing drug-resistance (Layer & McIntosh, 2006; Wang, Gao, Pettus, Phillips, & Bowersox, 2000; Wang, Pettus, Gao, Phillips, & Scott Bowersox, 2000). Along with preclinical utility in animal models, ω -conotoxin MVIIA has also been used in the clinic. Prior to its approval by the FDA for management of severe pain in patients'

refractory to other analgesic treatments, ω -conotoxin MVIIA was administered to 1200 patients in three double-blind, placebo-controlled studies and four open label long-term studies (Webster, Fakata, Charapata, Fisher, & Minehart, 2008). In another study, a double-blind, randomized trial in 111 cancer and AIDS patients with treatment refractory chronic pain, intrathecal ω -conotoxin MVIIA administration produced statistically significant and lasting analgesia. It is worth noting, however, that due to a narrow therapeutic window with inability to cross the blood-brain barrier and considerable side effects, ω -conotoxin MVIIA use is currently limited to intrathecal delivery in patients who have failed to respond to other treatments (Sanford, 2013).

At present, conotoxins are considered a combinatorial source for natural Structure Activity Relationship (SAR) candidates for producing small, non-peptide mimetics of conotoxins (Duggan & Tuck, 2015). As mentioned, several conotoxins have already passed the primary tests, with the best recognized commercial ω -conotoxin MVIIA approved by the FDA to treat intractable chronic pain in cancer and AIDS patients (Eisapoor, Jamili, Shahbazzadeh, Ghavam Mostafavi, & Pooshang, 2016; Rigo et al., 2013; Rigo et al., 2013). The utility of conotoxins for a wider range of applications has not only demonstrated their potential therapeutic value but has also stimulated more interest from biotech and pharmaceutical companies to support conotoxin research. In addition to ω -conotoxin MVIIA, other conopeptides are currently undergoing rigorous trials, with ω -conotoxin CVID successfully completing phase II trials, albeit with high cytotoxicity. It is important to stress that the clinical studies of ω -conotoxin MVIIA revealed significant side

effects such as gait disruptions, dizziness, nystagmus, confusion, somnolence, fever, postural hypotension, urinary retention, nausea and vomiting (Staats et al., 2004). Due to high medical relevance and research progress, increasing numbers of conopeptides are currently under investigation and validation as drug leads. Nevertheless, like other peptide drug candidates, the inability of these peptides to cross the blood-brain barrier remains a major challenge, urging research and discovery of more effective delivery means.

7. Beta-bungarotoxin

Since the pioneering research and isolation of crotoxin from the venom of *Crotalus durissus terrificus*, a wide range of snake venom proteins and peptide complexes have been identified and characterized (Slotta & Fraenkel-Conrat, 1938). Most of these have proven to be potent neurotoxins of various biochemical identity, including phospholipase A₂ (PLA2) or PLA2-like molecules, metalloproteases, serine proteases, C-type lectin related proteins (CLRPs) and three-finger toxins (3FTxs) with a wide range of biological activities (Doley & Kini, 2009; Koh, Armugam, & Jeyaseelan, 2006; Kwong, McDonald, Sigler, & Hendrickson, 1995). Over the past two decades, the effects of these molecules on different cell types has been investigated and characterized, with their pharmacological effects described at the neuromuscular junction. Among these, bungarotoxins (BuTx) isolated from the venom of different *Bungarus* species have been most thoroughly investigated, with the venom of *B. multicinctus* (Taiwan banded krait) best characterized (Fig. 6A). Based on electrophoretic mobility, the crude venom has

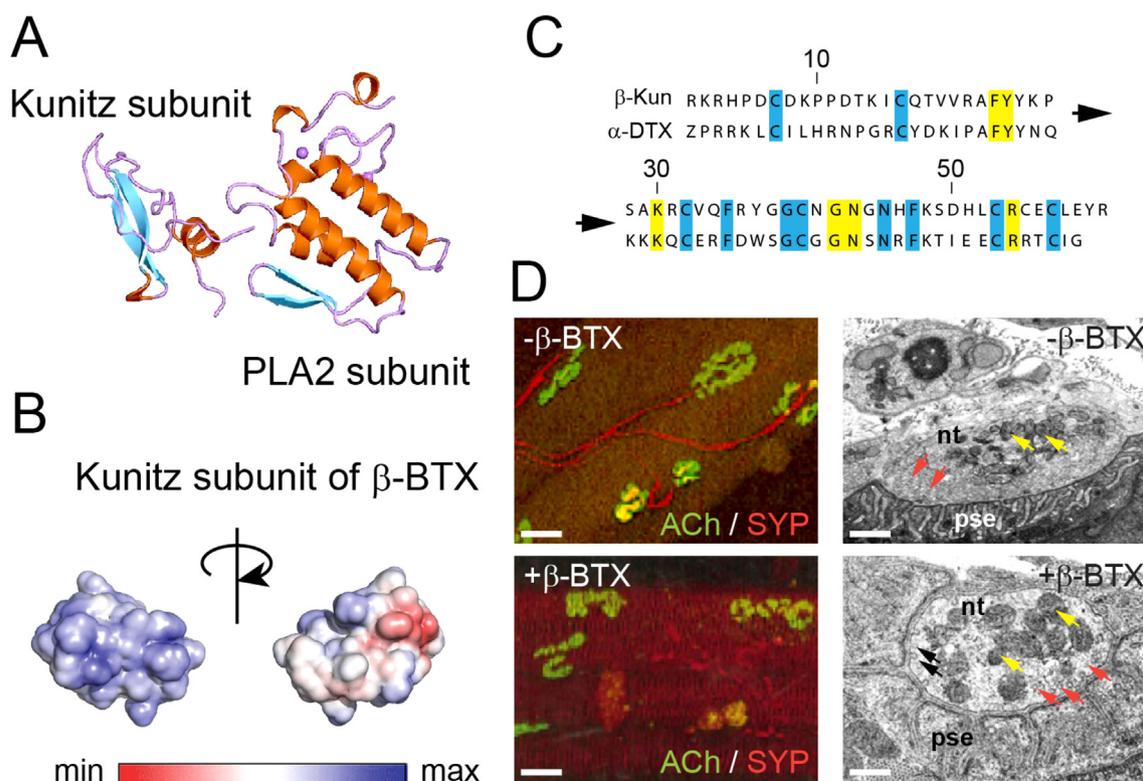


Fig. 6. Structure of β -bungarotoxin (BTX) and its inhibitory effects on presynaptic terminals. (A) 3D structure of β -BTX highlighting the Kunitz subunit implicated in ion channel binding and phospholipases 2 (PLA2) enzymatic subunit responsible for the paralytic effects of the venom on synaptic transmission. (B) Electrostatic potential of the Kunitz subunit of β -BTX B2 chain rendered in 3D on the solvent-accessible surface in PyMOL with surface electrostatic potential representation from two perspectives. Scale bar indicates the electrostatic potential ranging from -5000 to + 5000 kt/e. Negative regions - in red; positive regions - in blue; neutral regions - in gray. Adapted with permission from (Zupunski & Kordis, 2016). (C) Sequence alignment of β -Kun and α -DTX with conserved residues highlighted in blue and yellow. (D) Double neurofilament and ACh receptor labeling of the mouse muscle used to study the effects of β -BTX on the innervation of motor units in the skeletal muscles. Control preparation (- β -BTX) reveals a typical pattern of innervations of motor units by nerve endings. A preparation made 6 h after the inoculation with toxin (+ β -BTX) reveals the breakdown of the axon with however retained ACh receptors. Electron micrographs showing strong depletion of presynaptic vesicles at the neuromuscular junction by β -BTX, leading to blockade of synaptic transmission: yellow arrows - mitochondria; red arrows - clear synaptic vesicles filled with acetylcholine; black arrows - synaptic vesicle fusion profiles; nt - nerve terminal; pse - post synaptic element. Adapted with permission from (Dixon & Harris, 1999).

been separated into four distinct fractions, three toxic (α , β and γ) and one non-toxic (Kullmann, de Groat, & Artim, 2009). It has been reported that α -BuTx belongs to 3FTXs and binds to post-synaptic nicotinic receptors at the neuromuscular junction, while the β - and γ -BuTx belong to PLA2 and act at presynaptic terminals to reduce acetylcholine release (Chang, Chang, Wagner, & Chey, 1999; Halliwell & Dolly, 1982a, 1982b; Lewis & Garcia, 2003; Lewis & Gutmann, 2004). The initial studies have been followed by identification of an additional, κ -BuTx, also with a postsynaptic site of action. However, unlike α -BuTx, κ -BuTx shows little effect at the neuromuscular junction but is more specific to nicotinic acetylcholine receptor (nAChR) subunits enriched in postsynaptic compartments of central synapses (Kullmann et al., 2009). Currently, over one hundred different α -BuTx have been purified and tested as post-synaptic toxins, while β -BuTx are the most investigated proteases with a presynaptic site of action. Like many peptides isolated from snake venom, after entry into the circulatory system, BuTx targets the peripheral nerves and synapses, causing paralysis of skeletal muscles. The postsynaptic action mechanism of α -BuTx is rather straightforward and results from its competitive binding to postsynaptic nAChRs in skeletal muscles (Chang et al., 1999; Changeux, 1990; Fruchart-Gaillard, Mourier, Marquer, Menez, & Servent, 2006; Mulle & Changeux, 1990). At a molecular level, α -BuTx binds to the $\alpha 1$ nAChR, a mechanism that qualifies it as a curare-mimetic. In addition to peripheral synapses, α -BuTx is also capable of blocking neuronal cholinergic receptors in the central nervous system, but to a lesser extent.

As already noted, β -BuTx is a potent PLA2 complex with selective presynaptic site of action. Like other PLA2 enzymes, β -BuTx exists as a monomer and tends to interact with other PLA2 (or PLA2-like) molecules to form complexes, through covalent or non-covalent bonds (Fig. 6A). As a relatively large basic protein ($pI=9.5$), with a molecular weight of 21 800 kDa, β -BuTx is the only known covalent PLA2 (phospholipase A2) complex consisting of two dissimilar polypeptide chains - A and B, which are held together by a single disulfide bond (Kondo, Narita, & Lee, 1978; Kondo, Toda, & Narita, 1978). The A chain is homologous to Group I PLA2 enzymes, while the B chain is structurally homologous to BPTI, the so called Kunitz type of PLA2 serine proteinase inhibitors, showing similarities with dendrotoxins (Doley & Kini, 2009; Kondo, Toda, & Narita, 1978) (Fig. 6C). A number of isoforms of polypeptide A and B chains have been isolated and characterized, which are produced by the association of three different types of A chains and two different types of B moieties. The disruption of acetylcholine release at motor nerve terminals is critical for neurotoxicity and neuro-paralytic effects of β -BuTx, which typically becomes synergized by other active ingredients in the venom. As it is the case with other neurotoxins, synaptic transmission blockade caused by β -BuTx is a multistep process with initial reduction in acetylcholine release followed by transient enhancement and tailed by a profound inhibition, leading to complete neurotransmission block (Koh et al., 2006; Lewis & Gutmann, 2004; Sen, Grantham, & Cooper, 1976; Strong, Goerke, Oberg, & Kelly, 1976; Sun et al., 2010). The molecular processes underlying these stepwise changes remain controversial and involve functional as well as structural changes (Fig. 6D). The initial phase in β -BuTx appears to involve modulation of the presynaptic Ca^{2+} and K^+ currents, due to direct binding of β -BuTx. The interactions of β -BuTx with K^+ channels are thought to orient the PLA2 phospholipase chain of the toxin towards the membrane (Nicholls, Snelling, & Dolly, 1985; Tibbs, Dolly, & Nicholls, 1989). This step is followed by conformational changes of PLA2, which hydrolyses phospholipids in the external leaflet of the membrane leading to formation of an excess of phospholipids and fatty acids. These intermediates of lipolysis in turn promote exocytosis of synaptic vesicles, leading to gradual depletion of presynaptic terminals and neurotransmission blockade (Chen & Lee, 1970; Cull-Candy, Fohlman, Gustavsson, Lullmann-Rauch, & Thesleff, 1976; Rigoni et al., 2004; Rigoni et al., 2008). Increase in the cation conductivity of the presynaptic membrane and particularly Ca^{2+} -channels at this stage, transiently facilitates SNARE-mediated transmitter release. Interestingly,

β -BuTx also appears to enter into the nerve endings, a process that is very rapid and apparently depends on SNAREs but is not linked with ATP-ase activity (Montecucco & Rossetto, 2000).

Interaction of β -BuTx with cytosolic proteins has been demonstrated both *in vitro* and *in vivo*, which may be partly responsible for the specific association of β -BuTx with mitochondria, leading to the opening of mitochondrial pores (Rigoni et al., 2008) and activating the release of alarmins (Zornetta et al., 2012). Amplified phospholipase activity inside neurons is a crucial determinant of β -BuTx intoxication; hence, blockade of the uptake of this toxin and resultant of targeted phospholipolysis could be a very important stage for preventing impairments of neurotransmission. It has been shown that the phospholipase activity of β -BuTx binding to the specific sites of the mitochondrial membrane is essential in the opening of membranous pores, resulting in ATP deficit and mitochondrial degeneration (de Carvalho et al., 2014; Logonder et al., 2009; Rigoni et al., 2008). In addition to transient facilitation of transmitter release, an increase in intracellular Ca^{2+} concentration upregulates the activity of hydrolases, which cause slow-onset extensive damage and degeneration of nerve terminals (Fig. 6D). Overall, inhibition of synaptic transmission by β -BuTx is associated with loss of synaptic vesicles, mitochondrial damage and degeneration of motor nerve terminals, transient upregulation of voltage-gated Na^+ channels and a reduction in immunoreactivity of SNARE proteins, effects that have been largely attributed to the increased activity of PLA2 at presynaptic terminals (Vulfius et al., 2017). Despite that structural and functional alterations associated with β -BuTx intoxication can be severe, they are generally reversible, and as such can provide a useful experimental model for studying synaptic and nerve regeneration after damage or degeneration (Duregotti et al., 2015; Negro et al., 2017; Rigoni & Montecucco, 2017).

Despite the potency and selectivity of β -BuTx in targeting presynaptic functions at neuromuscular junctions, the therapeutic use of this peptide is hampered by toxicity-related degenerative changes of axons and nerve terminals. Nevertheless, the phospholipase activity of PLA2 from the β -BuTx B chain has shown great promise for its potent non-steroid anti-inflammatory effects. Moreover, the PLA2 inhibitor effects with specific affinities for various PLA2 enzymes have been shown to exert potent anti-enzymatic, anti-mycotic, anti-edema-inducing, anti-cytotoxic and anti-bacterial activities (Koh et al., 2006; Kullmann et al., 2009; Soares, Bellintani, Ribeiro, Tomassini, & Ribeiro dos Santos, 2003). In addition to the above-mentioned capabilities with their potential downstream effects on neuronal and synaptic processes, changes in the activity of endogenous PLA2 are thought to be crucial in inflammatory processes related to numerous acute and chronic neurological disorders accompanying neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases as well as brain tumours (Faroqui, Litsky, Faroqui, & Horrocks, 1999; Ross, Mszczynska, Erlich, & Kish, 1998; Sun et al., 2010). As treatment for these disorders with non-specific inhibitors has been of limited success, future research is required to better understand the biology of β -BuTx, to harvest its specific pharmacological effects for therapeutic benefit.

8. Dendrotoxins

Enriched in the venom of green mamba *Dendroaspis angusticeps*, DTXs have proven to be strong stimulators of both, neuromuscular junctions at the periphery and central synapses, causing hyperexcitability of axons with convulsive symptoms (Harvey, 2001). Structurally, dendrotoxins are a family of single-chain homologous polypeptides (Dolly & Parcej, 1996; Harvey & Karlsson, 1982; Parcej & Dolly, 1989; Strydom, 1973a) (Fig. 7A, B). Biochemical analysis with fractionation studies showed that DTXs represents a collection of small proteins, which are purified and categorized as α -DTX, β -DTX, γ -DTX and δ -DTX (Benishin, Sorensen, Brown, Krueger, & Blaustein, 1988). In addition to DTXs isolated from *D. angusticeps*, several related peptides have also been found in venoms of the Western green mamba and the Black

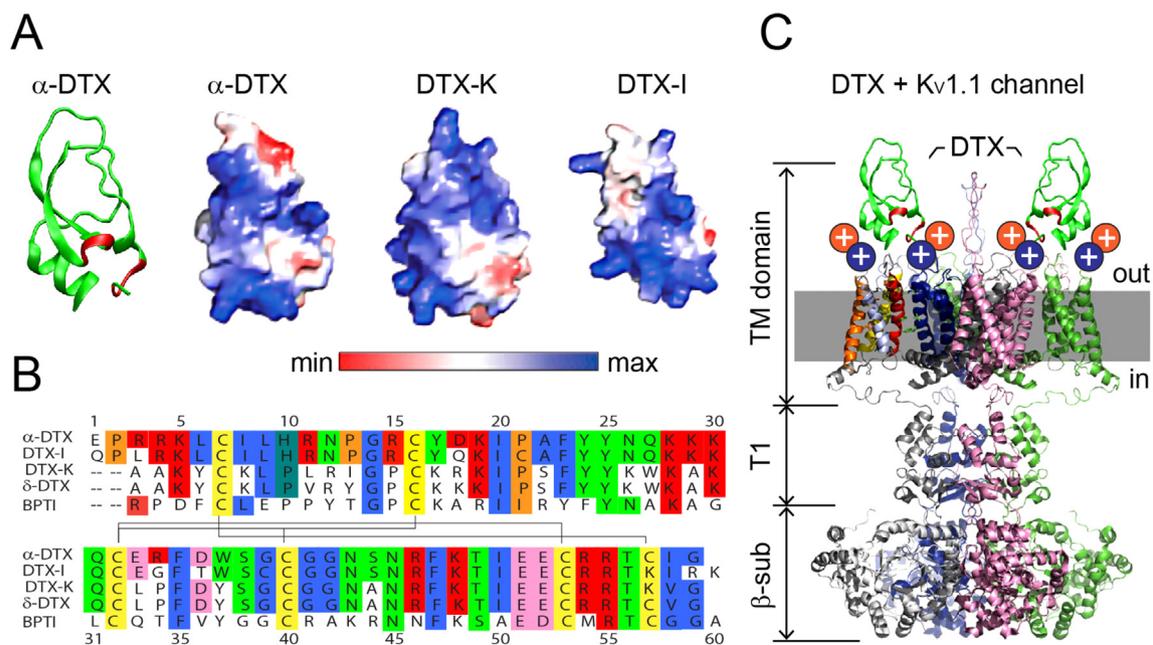


Fig. 7. Dendrotoxins: structure and pharmacological targets at presynaptic terminals. (A) Left: 3D structure of α -DTX; positively charged amino acid residues in the N-terminus and the β -turn region implicated in binding of potassium channels colored in red. Left-middle to right: surface electrostatic potential representation of α -DTX, DTX-K and DTX-I aligned, respectively. Negative regions - in red; positive regions - in blue; neutral regions - in gray. Scale bar indicates electrostatic potential ranging from -5000 to + 5000 kt/e. Adapted with permission from (Garcia-Fernandez et al., 2016). (B) Amino acid sequences of different dendrotoxin variants highlighting structural similarities and extent of evolutionary conservation, which produce comparable molecular architecture and folding conformation. α -DTX and bovine pancreatic trypsin inhibitor (BPTI) also show 35% sequence identity as well as identical disulfide bonds. Despite the structural homology, dendrotoxins do not appear to exhibit any measurable inhibitory protease activity. (C) Crystal structure of Kv1.1 α -subunit of potassium channels, with transmembrane (TM) domain, intracellular T1 domain and β -subunit. Note that the pore or Kv channels are formed by tetramerization of four α -subunits with four axillary β -subunits (colored in green, blue, grey and pink). Positively charged residues of DTX form bonds with negative extracellular residues shielding the pore of the channel and blocking the K^+ efflux. Adapted with permission from (Ovsepiyan, O'Leary, et al., 2016).

mamba (toxins I and K) (Harvey & Karlsson, 1982; Strydom, 1973b, 1973c). Three homologues of DTX were also isolated from the sea anemone *Anemonia sulcata* (Schweitz et al., 1995). Despite close similarity with Kunitz serine protease inhibitors, DTXs are very weak trypsin inhibitors. The high selectivity and binding of DTXs to K^+ channels played a pivotal role in purification and characterization of the pore forming α -subunit of mammalian K^+ channels (Dolly & Bagetta, 2002; Dolly & Parcej, 1996; Parcej & Dolly, 1989). Made of 57-60 amino acid residues cross-linked by three disulfide bridges, DTXs selectively bind α -subunits of several low-voltage activated Kv1 channels, the largest subfamily of K^+ channels. This reaction is driven by electrostatic interactions between the positively charged residues in the DTX cationic domain and negatively charged groups in the ion pore of the K^+ channel near the extracellular surface, leading to mechanical shielding of the channel pore (Harvey, 2001; Imredy & MacKinnon, 2000) (Fig. 7C). Through such process, DTXs inhibit A-type and slowly-inactivating K^+ currents in a variety of neuronal preparations and in heterologous systems (Bagchi et al., 2014; Gamkrelidze, Giaume, & Peusner, 1998; Ovsepiyan et al., 2013; Ovsepiyan, LeBerre, et al., 2016; Tytgat, Debont, Carmeliet, & Daenens, 1995).

Using ^{125}I DTX radiolabelling methods, immunoprecipitation and pharmacology, these toxin peptides were instrumental in defining the distribution of Kv1 channels throughout the nervous system and their biology. It has been demonstrated that Kv1 channels are integral membrane proteins composed of four identical or distinct α -subunits (homo- or hetero-tetramers respectively) that form the ion conductive pore, while associated with them four axillary β -subunits enable their trafficking and stabilization on the surface membrane (Dolly et al., 1994; Dolly & Parcej, 1996; Parcej & Dolly, 1989) (Fig. 7C). Pharmacological analysis and biophysical profiling of cloned DTX-sensitive K^+ channels showed that DTX-K is remarkably active on homo-tetramers of the Kv1.1 subunit as compared to any other member of the Kv1 channel family (Bagchi et al., 2014; Robertson, Owen, Stow, Butler,

& Newland, 1996). Detailed characterization of the affinity of DTXs for predominant Kv1 subunits showed a Kv1.1 < Kv1.2 < Kv1.6 relation (Dolly, Rettig, et al., 1994; Gutman et al., 2005; Scott et al., 1994; Wang et al., 1999; Wang, Parcej, & Dolly, 1999). Of note, all four subunits in the DTX-sensitive K^+ channel can interact simultaneously with the toxin, however binding of only one toxin molecule is sufficient to block the K^+ current. Acting upon channels expressed at presynaptic terminals of motor nerve endings, α -DTXs cause enhancement of acetylcholine release and muscular hyperactivity (Anderson & Harvey, 1988; Harvey, 2001, 2014). In the Ranvier nodes of frog sciatic nerves, DTX-I prolongs the duration of the action potential by blocking a fraction of the K^+ currents (Weller et al., 1985), with toxin-sensitive Kv1 channel subunits located primarily in the paranodal regions. Due to such a specialized location, the binding of DTX-I to the K^+ channel and its effects on action potentials are significantly enhanced in demyelinated axons of mouse models akin to multiple sclerosis (Bagchi et al., 2014). In sensory neurons, α -DTX produces repetitive action potential firing, which leads to the blockade of rapid-activating and slowly-inactivating K^+ currents (McAlexander & Undem, 2000; Stansfeld, Marsh, Parcej, Dolly, & Brown, 1987). In central neurons, α -DTX promotes repetitive action potential firing (Bagchi et al., 2014; Halliwell, Othman, Pelchen-Matthews, & Dolly, 1986; Ovsepiyan et al., 2013; Ovsepiyan, LeBerre, et al., 2016; Poulter, Hashiguchi, & Padjen, 1989) while DTX-I has been shown to augment long-term potentiation (Kondo et al., 1992). In projection neurons of the deep cerebellar nucleus, α -DTX was shown to dampen depolarizing inputs and attenuate rebound excitation (Ovsepiyan et al., 2013), while in upstream cerebellar Purkinje cells it enhances inhibitory postsynaptic currents via presynaptic effects (Southan & Robertson, 1998a, 1998b). Like at the neuromuscular junction, the enhancing effect of DTX on inhibitory transmission in Purkinje neurons is due to increased quantal content of synaptic release. The same mechanisms but acting at excitatory inputs underlies DTX-induced epileptiform hyperactivity when high

Box 1

Autoimmune disorders targeting presynaptic functions and causing neuromuscular paralysis

While mechanistically different from the paralysis caused by presynaptic neurotoxins, several autoantibodies targeting axons and nerve terminals can also cause acute or long-lasting weakness of neuromuscular synapses and motor deficit. Indeed, neuromuscular junction has been recognized as a veritable hot spot of several autoimmune diseases, which include myasthenia gravis (MG), Lambert-Eaton myasthenic syndrome (LEMS), acquired neuromyotonia (NMT) and Guillain-Barré syndrome (GBS). In case of MG, autoantibodies target the neuronal post-synaptic (and also pre-synaptic) acetylcholine receptors (AChR), while LEMS is caused by autoimmune response against presynaptic voltage-gated calcium and potassium channels. The NMT and GBS, on the other hand, are associated with anti-ganglioside antibodies, which cause acute inflammatory neuropathies, leading to impaired axonal conductivity and neuromuscular weakness (Lang & Vincent, 2009).

As the most common autoimmune disease of the NMJ, MG is an acquired condition, with over 80% cases having autoantibodies against the nAChR. When in excess, the autoantibodies cause strong reduction in the density of nAChR at NMJ via their enhanced internalization, accelerated degradation or complement-mediated damage of the post-synaptic membrane. In majority of MG cases, abnormalities of thymus glands have been documented, where production of antibodies takes place. It is thought that the expression of autoantigens in the thymic epithelial cells is responsible for the depletion of self-reactive T lymphocytes, which in turn are responsible for MG specific antibody synthesis. Although questions remain over the mechanisms the abnormal autoimmune response, these antibodies, when released in the circulatory liquids, target nAChR rich postsynaptic folds of NMJ, leading to development of the fatigable muscle weakness (Leite et al., 2008). Similar to MG, LEMS also causes proximal muscle weakness of autoimmune origin, but through targeting presynaptic voltage-gated Ca^{2+} channels. In LEMS, pathogenic antibodies against these channels have been found in over 85% of patients (Lang & Vincent, 2009). In stark contrast to MG, LEMS is a prototypic paraneoplastic disorder, with the majority of cases associated with small cell lung carcinomas (SCLC). It is of interest to note that SCLC patients with clinical LEMS have shown better prognosis than patients with SCLC without neurological dysfunctions, perhaps due to the fact that the neurological symptoms assist the early tumor diagnosis (Maddison, Newsom-Davis, Mills, & Souhami, 1999; Wirtz et al., 2005). Some data also suggest differences in voltage-gated Ca^{2+} channel antibodies between the tumor and non-tumor patients, with antibodies in the absence of tumors binding the extracellular domain IV of the α -1A subunit (Pellkofer et al., 2008). Future research and identification of new antibodies and analysis of the clinical history holds promise for an earlier and accurate diagnosis of SCLC in LEMS patients with improved treatment.

Unlike MG and LEMS, muscle weakness and neuromuscular paralysis in NMT and GBS are typically precipitated by an acute inflammation, which follows by autoimmune response leading to impaired axonal conductivity and disintegration of the membrane integrity at the NMJ. Known also as Isaac's syndrome, NMT is characterised by muscle twitching (myokymia), cramps, muscle stiffness and excessive sweating. In most severe cases, NMT patients also reveal additional central symptoms, which include sleep disorders, memory loss and anxiety. In ~ 40% NMT

patients, antibodies to voltage-gated potassium channels are detected from the very early stages of the disease (Hart, Maddison, Newsom-Davis, Vincent, & Mills, 2002). For detection of the expression levels of voltage-gated potassium channels, ^{125}I -labelled DTX-based test are used, which binds to $K_v1.1$, 1.2 and 1.6 subunits of Shaker family channels expressed presynaptic terminals. At present, it remains unclear which subunit of potassium channel is most susceptible to the antibody and if the neurological signs depend on the channel type. Although at this stage, the aetiology of the disease remains largely unknown, the disease seems to be a multifactorial. The majority of cases seem to emerge and unfold as a self-limiting monophasic disease, while in ~ 20% cases thymic tumours have been documented (Lang & Vincent, 2009). The final most studies autoimmune disorder affecting neuromuscular transmission is GBS and its variants, which are acute autoimmune-mediated neuropathies that cause demyelination of peripheral nerves and axonal damage. Typically, these conditions are precipitated by acute infection involving *Campylobacter jejuni* in the gastrointestinal tract or respiratory infection. In the acute phase of the disease, autoantibodies against gangliosides including mono-sialyl-GM1 (GBS) or polysialyl-GD1B have been found in between 20 to 60% of patients. These antibodies can bind to the motor nerve terminal, causing changes in neurotransmitter release (Willison, 2005). The levels of antibodies against GD1a/GD1b and GD1b/GT 1b in the membrane have been shown especially strongly correlating with the severe forms of GBS (Kusunoki, Kaida, & Ueda, 2008). It is important to note that both, the inducing pathogens and the autoantibody isotypes may be indicative of the severity of the neurological signs and prognosis. For instance, patients with cross-reactive *C. jejuni* IgG1 and IgA anti-ganglioside antibodies typically show poor recovery to immunotherapy, whilst those having IgG1 and IgG3 with negative *C. jejuni* serology but reactive to *H. influenza*, had a better prognosis (Jacobs et al., 2008).

Overall, despite some shared targets and clinical manifestation with neurotoxins targeting presynaptic terminals, the aetiology, pathobiological mechanisms and treatment strategies autoimmune disorders targeting NMJ fundamentally differ from those induced by neurotoxins. In addition to symptomatic therapies, depending on their forms and stage, the NMJ weakness caused by autoimmune response require intense and occasionally long-term immune-suppressant therapies combined with non-steroid anti-inflammatory treatment (Skeie et al., 2006). Development of new immunosuppressive drugs are currently in trials, and proved effective in some MG cases, while autoantibodies depleting B-cells lymphocytes or combinatorial therapies have shown promises in MG and GBS preclinical trials (Skeie et al., 2006; Willison, Jacobs, & van Doorn, 2016). Whether these approaches could be modified using humanized antibodies for clinical applications remains to be determined by future studies.

specific peptides and proteins have been developed and fine-tuned by microbes, plants and animals as part of their defensive or offensive system. In light of the critical importance of neuronal functions for animal adaptation and survival, it is hardly surprising that many highly potent biological toxins and pathogens became specialised for targeting presynaptic functions, which play a major role in neuronal integration and motor functions. Indeed, the dynamic and complex processes involving interaction of supramolecular scaffolds driving transmitter release at presynaptic terminals present an extremely attractive and vulnerable target. Throughout this review, we presented a concise summary of the biology and function of several well-characterized neurotoxins that target the presynaptic release machinery (Fig. 8), along with the brief overview of autoimmune disease also targeting this

highly complex specialization of neurons (see Box 1). We discussed their shared features as well as the differences and highlighted numerous unique characteristics which present major interest for basic and translational neurosciences. As it emerges, the extraordinary wealth of neurotoxins produced from bacteria to vertebrates, presents invaluable research tools as well as potential leads for developing new drugs affecting transmitter release machinery and a wide range of neuronal regulatory mechanisms. Despite their vast natural diversity and origin, neurotoxins acting at the presynaptic terminals appear to converge on a few selected processes and functions. The extraordinary efficacy of neurotoxins and their selectivity for specific functional processes, render presynaptic neurotoxins as an unique source of new bio-therapeutics. Notwithstanding the safety concerns and methodical challenges, an increasing number of peptides derived from bacterial toxins and animal venoms targeting synaptic functions have passed the test of time as bio-therapeutic candidates, contributing assertively in pre-clinical studies of animal models, thus paving a new way for their future clinical usage.

Conflict to interest

The authors claim no conflict of interest.

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References

- Adams, D. J., & Berecki, G. (2013). Mechanisms of conotoxin inhibition of N-type (Ca_v2.2) calcium channels. *BBA-Biomembranes* 1828, 1619–1628. <https://doi.org/10.1016/j.bbmem.2013.01.019>.
- Adams, M. E. (2004). Agatoxins: Ion channel specific toxins from the American funnel web spider, *Agelenopsis aperta*. *Toxicon: Official Journal of the International Society on Toxinology* 43, 509–525. <https://doi.org/10.1016/j.toxicon.2004.02.004>.
- Al-Sabi, A., Daly, D., Hoefler, P., Kinsella, G. K., Metais, C., Pickering, M., et al. (2017). A rational design of a selective inhibitor for Kv1.1 channels prevalent in demyelinated nerves that improves their impaired axonal conduction. *Journal of Medicinal Chemistry* 60, 2245–2256.
- Anderson, A. J., & Harvey, A. L. (1988). Effects of the potassium channel blocking dendrotoxins on acetylcholine release and motor nerve terminal activity. *British Journal of Pharmacology* 93, 215–221.
- Andreu, A., Fairweather, N., & Miller, A. D. (2008). Clostridium neurotoxin fragments as potential targeting moieties for liposomal gene delivery to the CNS. *Chembiochem: A European Journal of Chemical Biology* 9, 219–231. <https://doi.org/10.1002/cbic.200700277>.
- Aoki, K. R. (2003). Evidence for antinociceptive activity of botulinum toxin type A in pain management. *Headache* 43(Suppl. 1), S9–15.
- Ashton, A. C., & Dolly, J. O. (1988). Characterization of the inhibitory action of botulinum neurotoxin type A on the release of several transmitters from rat cerebrocortical synaptosomes. *Journal of Neurochemistry* 50, 1808–1816.
- Ashton, A. C., & Dolly, J. O. (2000). A late phase of exocytosis from synaptosomes induced by elevated [Ca²⁺]_i is not blocked by Clostridial neurotoxins. *Journal of Neurochemistry* 74, 1979–1988.
- Ashton, A. C., Volynski, K. E., Lelianova, V. G., Orlova, E. V., Van Renterghem, C., Canepari, M., et al. (2001). alpha-Latrotoxin, acting via two Ca²⁺-dependent pathways, triggers exocytosis of two pools of synaptic vesicles. *The Journal of Biological Chemistry* 276, 44695–44703. <https://doi.org/10.1074/jbc.M108088200>.
- Bade, S., Rummel, A., Reisinger, C., Karnath, T., Ahnert-Hilger, G., Bigalke, H., et al. (2004). Botulinum neurotoxin type D enables cytosolic delivery of enzymatically active cargo proteins to neurones via unfolded translocation intermediates. *Journal of Neurochemistry* 91, 1461–1472. <https://doi.org/10.1111/j.1471-4159.2004.02844.x>.
- Bagchi, B., Al-Sabi, A., Kaza, S., Scholz, D., O'Leary, V. B., Dolly, J. O., et al. (2014). Disruption of myelin leads to ectopic expression of K(V)1.1 channels with abnormal conductivity of optic nerve axons in a cuprizone-induced model of demyelination. *PLoS One* 9, e87736.
- Bagetta, G., Nair, S., Nistico, G., & Dolly, J. O. (1994). Hippocampal damage produced in rats by alpha-dendrotoxin—A selective K⁺ channel blocker—Involves non-NMDA receptor activation. *Neurochemistry International* 24, 81–90.
- Barnett, D. W., Liu, J., & Misler, S. (1996). Single-cell measurements of quantal secretion induced by alpha-latrotoxin from rat adrenal chromaffin cells: Dependence on extracellular Ca²⁺. *Pflügers Archives: European Journal of Physiology* 432, 1039–1046.
- Benecke, R., Takano, K., Schmidt, J., & Henatsch, H. D. (1977). Tetanus toxin induced actions on spinal Renshaw cells and Ia-inhibitory interneurons during development of local tetanus in the cat. *Experimental Brain Research* 27, 271–286.
- Benishin, C. G., Sorensen, R. G., Brown, W. E., Krueger, B. K., & Blaustein, M. P. (1988). Four polypeptide components of green mamba venom selectively block certain potassium channels in rat brain synaptosomes. *Molecular Pharmacology* 34, 152–159.
- Bentivoglio, A. R., Fasano, A., Ialongo, T., Soletti, F., Lo Fermo, S., & Albanese, A. (2009). Outcome predictors, efficacy and safety of Botox and Dysport in the long-term treatment of hemifacial spasm. *European Journal of Neurology* 16, 392–398. <https://doi.org/10.1111/j.1468-1331.2008.02507.x>.
- Beraud, E., & Chandy, K. G. (2011). Therapeutic potential of peptide toxins that target ion channels. *Inflammation & Allergy Drug Targets* 10, 322–342.
- Bercsenyi, K., Schmieg, N., Bryson, J. B., Wallace, M., Caccin, P., Golding, M., et al. (2014). Tetanus toxin entry. Nidogens are therapeutic targets for the prevention of tetanus. *Science* 346, 1118–1123. <https://doi.org/10.1126/science.1258138>.
- Binz, T., Kurazono, H., Wille, M., Frevert, J., Wernars, K., & Niemann, H. (1990). The complete sequence of botulinum neurotoxin type A and comparison with other clostridial neurotoxins. *The Journal of Biological Chemistry* 265, 9153–9158.
- Bixel, M. G., Weise, C., Bolognesi, M. L., Rosini, M., Brierly, M. J., Mellor, I. R., et al. (2001). Location of the polyamine binding site in the vestibule of the nicotinic acetylcholine receptor ion channel. *The Journal of Biological Chemistry* 276, 6151–6160. <https://doi.org/10.1074/jbc.M008467200>.
- Black, J. D., & Dolly, J. O. (1986). Interaction of 125I-labeled botulinum neurotoxins with nerve terminals. I. Ultrastructural autoradiographic localization and quantitation of distinct membrane acceptors for types A and B on motor nerves. *The Journal of Cell Biology* 103, 521–534.
- Black, J. D., & Dolly, J. O. (1987). Selective location of acceptors for botulinum neurotoxin A in the central and peripheral nervous systems. *Neuroscience* 23, 767–779.
- Blasi, J., Chapman, E. R., Link, E., Binz, T., Yamasaki, S., De Camilli, P., et al. (1993). Botulinum neurotoxin A selectively cleaves the synaptic protein SNAP-25. *Nature* 365, 160–163. <https://doi.org/10.1038/365160a0>.
- Boland, L. M., Morrill, J. A., & Bean, B. P. (1994). omega-Conotoxin block of N-type calcium channels in frog and rat sympathetic neurons. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 14, 5011–5027.
- Bomba-Warczak, E., Vevea, J. D., Brittain, J. M., Figueroa-Bernier, A., Tepp, W. H., Johnson, E. A., et al. (2016). Interneuronal transfer and distal action of tetanus toxin and botulinum neurotoxins A and D in central neurons. *Cell Reports* 16, 1974–1987. <https://doi.org/10.1016/j.celrep.2016.06.104>.
- Bourinet, E., & Zamponi, G. W. (2017). Block of voltage-gated calcium channels by peptide toxins. *Neuropharmacology* 127, 109–115. <https://doi.org/10.1016/j.neuropharm.2016.10.016>.
- Brooks, V. B., Curtis, D. R., & Eccles, J. C. (1957). The action of tetanus toxin on the inhibition of motoneurons. *The Journal of Physiology* 135, 655–672.
- Brunger, A. T., & Rummel, A. (2009). Receptor and substrate interactions of clostridial neurotoxins. *Toxicon: Official Journal of the International Society on Toxinology* 54, 550–560. <https://doi.org/10.1016/j.toxicon.2008.12.027>.
- Capogna, M., Gähwiler, B. H., & Thompson, S. M. (1996). Calcium-independent actions of alpha-latrotoxin on spontaneous and evoked synaptic transmission in the hippocampus. *Journal of Neurophysiology* 76, 3149–3158. <https://doi.org/10.1152/jn.1996.76.5.3149>.
- Capogna, M., McKinney, R. A., O'Connor, V., Gähwiler, B. H., & Thompson, S. M. (1997). Ca²⁺ or Sr²⁺ partially rescues synaptic transmission in hippocampal cultures treated with botulinum toxin A and C, but not tetanus toxin. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 17, 7190–7202.
- de Carvalho, N. D., Garcia, R. C., Ferreira, A. K., Batista, D. R., Cassola, A. C., Maria, D., et al. (2014). Neurotoxicity of coral snake phospholipase A2 in cultured rat hippocampal neurons. *Brain Research* 1552, 1–16. <https://doi.org/10.1016/j.brainres.2014.01.008>.
- Ceccarelli, B., Hurlbut, W. P., & Iezzi, N. (1988). Effect of alpha-latrotoxin on the frog neuromuscular junction at low temperature. *The Journal of Physiology* 402, 195–217.
- Chaddock, J. A., & Marks, P. M. (2006). Clostridial neurotoxins: Structure-function led design of new therapeutics. *Cellular and Molecular Life Sciences* 63, 540–551. <https://doi.org/10.1007/s00018-005-5505-5>.
- Chaddock, J. A., Purkiss, J. R., Duggan, M. J., Quinn, C. P., Shone, C. C., & Foster, K. A. (2000). A conjugate composed of nerve growth factor coupled to a non-toxic derivative of Clostridium botulinum neurotoxin type A can inhibit neurotransmitter release in vitro. *Growth Factors* 18, 147–155.
- Chaddock, J. A., Purkiss, J. R., Friis, L. M., Broadbridge, J. D., Duggan, M. J., Fooks, S. J., et al. (2000). Inhibition of vesicular secretion in both neuronal and nonneuronal cells by a retargeted endopeptidase derivative of Clostridium botulinum neurotoxin type A. *Infection and Immunity* 68, 2587–2593.
- Chang, T. M., Chang, C. H., Wagner, D. R., & Chey, W. Y. (1999). Porcine pancreatic phospholipase A2 stimulates secretin release from secretin-producing cells. *The Journal of Biological Chemistry* 274, 10758–10764.
- Chang, T. M., Lee, K. Y., Chang, C. H., Li, P., Song, Y., Roth, F. L., et al. (1999). Purification of two secretin-releasing peptides structurally related to phospholipase A2 from canine pancreatic juice. *Pancreas* 19, 401–405.
- Changeux, J. P. (1990). The TiPS lecture. The nicotinic acetylcholine receptor: An allosteric protein prototype of ligand-gated ion channels. *Trends in Pharmacological Sciences* 11, 485–492.
- Chen, I. L., & Lee, C. Y. (1970). Ultrastructural changes in the motor nerve terminals caused by beta-bungarotoxin. *Virchows Archiv B, Cell Pathology* 6, 318–325.
- Cochran, S. M. (1998). *Ph.D. Thesis*. Glasgow: University of Strathclyde.
- Cochran, S. M., & Pratt, J. A. (1997). Regionally selective changes in the displacement of alpha-dendrotoxin binding by charybdotoxin and toxin K in the rat

- septohippocampal pathway during ageing. *British Journal of Pharmacology* 122 (U50-U).
- Cohen, C. J., Ertel, E. A., Smith, M. M., Venema, V. J., Adams, M. E., & Leibowitz, M. D. (1992). High affinity block of myocardial L-type calcium channels by the spider toxin omega-Aga-toxin IIIA: Advantages over 1,4-dihydropyridines. *Molecular Pharmacology* 42, 947–951.
- Coleman, M. H., Yamaguchi, S., & Rogawski, M. A. (1992). Protection against dendrotoxin-induced clonic seizures in mice by anticonvulsant drugs. *Brain Research* 575, 138–142.
- Cook, T. M., Protheroe, R. T., & Handel, J. M. (2001). Tetanus: A review of the literature. *British Journal of Anaesthesia* 87, 477–487.
- Craven, C. J., & Dawson, D. J. (1973). The chain composition of tetanus toxin. *Biochimica et Biophysica Acta* 317, 277–285.
- Creager, M. A., & Roddy, M. A. (1994). Effect of captopril and enalapril on endothelial function in hypertensive patients. *Hypertension* 24, 499–505.
- Cull-Candy, S. G., Fohlman, J., Gustavsson, D., Lullmann-Rauch, R., & Thesleff, S. (1976). The effects of taipoxin and netoxin on the function and fine structure of the murine neuromuscular junction. *Neuroscience* 1, 175–180.
- Daly, N. L., & Craik, D. J. (2009). Structural studies of conotoxins. *IUBMB Life* 61, 144–150. <https://doi.org/10.1002/iub.158>.
- Davletov, B. A., Meunier, F. A., Ashton, A. C., Matsushita, H., Hirst, W. D., Lelianova, V. G., et al. (1998). Vesicle exocytosis stimulated by alpha-latrotoxin is mediated by latrophilin and requires both external and stored Ca²⁺. *The EMBO Journal* 17, 3909–3920. <https://doi.org/10.1093/emboj/17.14.3909>.
- Davletov, B. A., Shamotienko, O. G., Lelianova, V. G., Grishin, E. V., & Ushkaryov, Y. A. (1996). Isolation and biochemical characterization of a Ca²⁺-independent alpha-latrotoxin-binding protein. *The Journal of Biological Chemistry* 271, 23239–23245.
- De Potter, W. P., Partoens, P., Schoups, A., Llona, I., & Coen, E. P. (1997). Noradrenergic neurons release both noradrenaline and neuropeptide Y from a single pool: The large dense cored vesicles. *Synapse* 25, 44–55.
- Deak, F., Liu, X., Khvotchev, M., Li, G., Kavalali, E. T., Sugita, S., et al. (2009). Alpha-latrotoxin stimulates a novel pathway of Ca²⁺-dependent synaptic exocytosis independent of the classical synaptic fusion machinery. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 29, 8639–8648.
- Dean, C., & Dresbach, T. (2006). Neuroligins and neuroligins: Linking cell adhesion, synapse formation and cognitive function. *Trends in Neurosciences* 29, 21–29. <https://doi.org/10.1016/j.tins.2005.11.003>.
- Devaux, J., Beeton, C., Beraud, E., & Crest, M. (2004). Ion channels and demyelination: Basis of a treatment of experimental autoimmune encephalomyelitis (EAE) by potassium channel blockers. *Revue Neurologique* 160, S16–S27.
- Diaz, A., & Dickenson, A. H. (1997). Blockade of spinal N- and P-type, but not L-type, calcium channels inhibits the excitability of rat dorsal horn neurones produced by subcutaneous formalin inflammation. *Pain* 69, 93–100.
- Dillingham, T. R. (2007). Musculoskeletal rehabilitation: Current understandings and future directions. *American Journal of Physical Medicine & Rehabilitation* 86, S19–S28.
- Dixon, R. W., & Harris, J. B. (1999). Nerve terminal damage by beta-bungarotoxin: Its clinical significance. *American Journal of Pathology* 154, 447–455.
- Dobrenis, K., Joseph, A., & Rattazzi, M. C. (1992). Neuronal lysosomal enzyme replacement using fragment C of tetanus toxin. *Proceedings of the National Academy of Sciences of the United States of America* 89, 2297–2301.
- Doley, R., & Kini, R. M. (2009). Protein complexes in snake venom. *Cellular and Molecular Life Sciences* 66, 2851–2871. <https://doi.org/10.1007/s00018-009-0050-2>.
- Dolly, J., O'Leary, V., & Lawrence, G. (2014). *S. O. Pharmacology of botulinum neurotoxins: Exploitation of their multifunctional activities as transmitter release inhibitors and neuron-targeted delivery vehicles*. New York: Springer.
- Dolly, J. O., & Bagetta, G. (2002). *Dendrotoxins from mamba snakes*. Totowa, NJ: Humana Press.
- Dolly, J. O., Black, J., Williams, R. S., & Mellings, J. (1984). Acceptors for botulinum neurotoxin reside on motor nerve terminals and mediate its internalization. *Nature* 307, 457–460.
- Dolly, J. O., de Paiva, A., Foran, P., Lawrence, G., Daniels-Holgate, P. U., & Ashton, A. G. (1994). Probing the process of transmitter release with botulinum and tetanus neurotoxins. *Seminars in Neuroscience* 6, 149–158.
- Dolly, J. O., Lawrence, G. W., Meng, J., Wang, J., & Ovsepian, S. V. (2009). Neuro-exocytosis: Botulinum toxins as inhibitory probes and versatile therapeutics. *Current Opinion in Pharmacology* 9, 326–335. <https://doi.org/10.1016/j.coph.2009.03.004>.
- Dolly, J. O., & O'Connell, M. A. (2012). Neurotherapeutics to inhibit exocytosis from sensory neurons for the control of chronic pain. *Current Opinion in Pharmacology* 12, 100–108. <https://doi.org/10.1016/j.coph.2011.11.001>.
- Dolly, J. O., & Parcej, D. N. (1996). Molecular properties of voltage-gated K⁺ channels. *Journal of Bioenergetics and Biomembranes* 28, 231–253.
- Dolly, J. O., Rettig, J., Scott, V. E., Parcej, D. N., Wittkat, R., Sewing, S., et al. (1994). Oligomeric and subunit structures of neuronal voltage-sensitive K⁺ channels. *Biochemical Society Transactions* 22, 473–478.
- Dolly, O. (2003). Synaptic transmission: Inhibition of neurotransmitter release by botulinum toxins. *Headache* 43(Suppl. 1), S16–S24.
- Duan, Z. G., Yan, X. J., He, X. Z., Zhou, H., Chen, P., Cao, R., et al. (2006). Extraction and protein component analysis of venom from the dissected venom glands of *Latrodectus tredecimguttatus*. *Comparative Biochemistry and Physiology Part B, Biochemistry & Molecular Biology* 145, 350–357. <https://doi.org/10.1016/j.cbpb.2006.08.006>.
- Duggan, M. J., Quinn, C. P., Chaddock, J. A., Purkiss, J. R., Alexander, F. C., Doward, S., et al. (2002). Inhibition of release of neurotransmitters from rat dorsal root ganglia by a novel conjugate of a Clostridium botulinum toxin A endopeptidase fragment and Erythrina cristagalli lectin. *The Journal of Biological Chemistry* 277, 34846–34852. <https://doi.org/10.1074/jbc.M202902200>.
- Duggan, P. J., & Tuck, K. L. (2015). Bioactive mimetics of conotoxins and other venom peptides. *Toxins* 7, 4175–4198. <https://doi.org/10.3390/toxins7104175>.
- Duregotti, E., Zanetti, G., Scorsetto, M., Megighian, A., Montecucco, C., Pirazzini, M., et al. (2015). Snake and spider toxins induce a rapid recovery of function of botulinum neurotoxin paralysed neuromuscular junction. *Toxins* 7, 5322–5336. <https://doi.org/10.3390/toxins7124887>.
- Edupuganti, O. P., Ovsepian, S. V., Wang, J., Zurawski, T. H., Schmidt, J. J., Smith, L., et al. (2012). Targeted delivery into motor nerve terminals of inhibitors for SNARE-cleaving proteases via liposomes coupled to an atoxic botulinum neurotoxin. *The FEBS Journal* 279, 2555–2567. <https://doi.org/10.1111/j.1742-4658.2012.08638.x>.
- Eisapoor, S. S., Jamili, S., Shahbazzadeh, D., Ghavam Mostafavi, P., & Pooshang, B. K. (2016). A new, high yield, rapid, and cost-effective protocol to deprotection of cysteine-rich conopeptide, omega-conotoxin MVIIA. *Chemical Biology & Drug Design* 87, 687–693. <https://doi.org/10.1111/cbdd.12702>.
- Elia, A. E., Filippini, G., Calandrella, D., & Albanese, A. (2009). Botulinum neurotoxins for post-stroke spasticity in adults: A systematic review. *Movement Disorders: Official Journal of the Movement Disorder Society* 24, 801–812. <https://doi.org/10.1002/mds.22452>.
- Ellinor, P. T., Zhang, J. F., Horne, W. A., & Tsien, R. W. (1994). Structural determinants of the blockade of N-type calcium channels by a peptide neurotoxin. *Nature* 372, 272–275. <https://doi.org/10.1038/372272a0>.
- Emsley, P., Fotinou, C., Black, I., Fairweather, N. F., Charles, I. G., Watts, C., et al. (2000). The structures of the H(C) fragment of tetanus toxin with carbohydrate subunit complexes provide insight into ganglioside binding. *The Journal of Biological Chemistry* 275, 8889–8894.
- Escoubas, P., & King, G. F. (2009). Venomics as a drug discovery platform. *Expert Review of Proteomics* 6, 221–224. <https://doi.org/10.1586/epr.09.45>.
- Fabbri, A., Travaglione, S., Falzano, L., & Fiorentini, C. (2008). Bacterial protein toxins: Current and potential clinical use. *Current Medicinal Chemistry* 15, 1116–1125.
- Farooqui, A. A., Litsky, M. L., Farooqui, T., & Horrocks, L. A. (1999). Inhibitors of intracellular phospholipase A2 activity: Their neurochemical effects and therapeutic importance for neurological disorders. *Brain Research Bulletin* 49, 139–153.
- Fezza, J. P., Howard, J., Wiley, R., Wesley, R. E., Klippenstein, K., & Dettbarn, W. (2000). The effects of tetanus toxin on the orbicularis oculi muscle. *Ophthalmic Plastic and Reconstructive Surgery* 16, 101–113.
- Fishman, P. S. (2009). *Tetanus toxin*. Elsevier.
- Fishman, P. S., & Carrigan, D. R. (1987). Retrograde transneuronal transfer of the C-fragment of tetanus toxin. *Brain Research* 406, 275–279.
- Fishman, P. S., & Carrigan, D. R. (1988). Motoneuron uptake from the circulation of the binding fragment of tetanus toxin. *Archives of Neurology* 45, 558–561.
- Fleck-Derderian, S., Shankar, M., Rao, A. K., Chatham-Stephens, K., Adjei, S., Sobel, J., et al. (2017). The epidemiology of foodborne botulism outbreaks: A systematic review. *Clinical Infectious Diseases* 66, S73–S81. <https://doi.org/10.1093/cid/cix846>.
- Foran, P., Lawrence, G. W., Shone, C. C., Foster, K. A., & Dolly, J. O. (1996). Botulinum neurotoxin C1 cleaves both syntaxin and SNAP-25 in intact and permeabilized chromaffin cells: Correlation with its blockade of catecholamine release. *Biochemistry* 35, 2630–2636. <https://doi.org/10.1021/bi9519009>.
- Foran, P. G., Davletov, B., & Meunier, F. A. (2003). Getting muscles moving again after botulinum toxin: Novel therapeutic challenges. *Trends in Molecular Medicine* 9, 291–299.
- Foran, P. G., Mohammed, N., Lisk, G. O., Nagwaney, S., Lawrence, G. W., Johnson, E., et al. (2003). Evaluation of the therapeutic usefulness of botulinum neurotoxin B, C1, E, and F compared with the long lasting type A. Basis for distinct durations of inhibition of exocytosis in central neurons. *The Journal of Biological Chemistry* 278, 1363–1371. <https://doi.org/10.1074/jbc.M209821200>.
- Francis, J. W., Bastia, E., Matthews, C. C., Parks, D. A., Schwarzschild, M. A., Brown, R. H., Jr., et al. (2004). Tetanus toxin fragment C as a vector to enhance delivery of proteins to the CNS. *Brain Research* 1011, 7–13. <https://doi.org/10.1016/j.brainres.2004.03.007>.
- Francis, J. W., Brown, R. H., Jr., Figueiredo, D., Remington, M. P., Castillo, O., Schwarzschild, M. A., et al. (2000). Enhancement of diphtheria toxin potency by replacement of the receptor binding domain with tetanus toxin C-fragment: A potential vector for delivering heterologous proteins to neurons. *Journal of Neurochemistry* 74, 2528–2536.
- Fruchart-Gaillard, C., Mourier, G., Marquer, C., Menez, A., & Servent, D. (2006). How three-finger-fold toxins interact with various cholinergic receptors. *Journal of Molecular Neuroscience* 30, 7–8. <https://doi.org/10.1385/JMN:30:1:7>.
- Gamkrelidze, G., Giaume, C., & Peusner, K. D. (1998). The differential expression of low-threshold sustained potassium current contributes to the distinct firing patterns in embryonic central vestibular neurons. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 18, 1449–1464.
- Garb, J. E., & Hayashi, C. Y. (2013). Molecular evolution of alpha-latrotoxin, the exceptionally potent vertebrate neurotoxin in black widow spider venom. *Molecular Biology and Evolution* 30, 999–1014. <https://doi.org/10.1093/molbev/mst011>.
- Garcia-Fernandez, R., Peigneur, S., Pons, T., Alvarez, C., Gonzalez, L., Chavez, M. A., et al. (2016). The kunitz-type protein SHP1-1 inhibits serine proteases and voltage-gated potassium channels. *Toxins* 8, 110. <https://doi.org/10.3390/toxins8040110>.
- Gejl, K. D., Ortenblad, N., Andersson, E., Plomgaard, P., Holmgren, H. C., & Nielsen, J. (2017). Local depletion of glycogen with supramaximal exercise in human skeletal muscle fibres. *The Journal of Physiology* 595, 2809–2821. <https://doi.org/10.1113/jp273109>.
- Ghazaryan, N. A., Ghulikian, L. A., Kishmiryan, A. V., Kirakosyan, G. R., Nazaryan, O. H., Ghevondyan, T. H., et al. (2015). Anti-tumor effect investigation of obtustatin and crude *Macrovipera lebetina obtusa* venom in S-180 sarcoma bearing mice. *European Journal of Pharmacology* 764, 340–345. <https://doi.org/10.1016/j.ejphar.2015.07.011>.
- Goodough, M. C., Oyler, G., Fishman, P. S., Johnson, E. A., Neale, E. A., Keller, J. E., et al. (2002). Development of a delivery vehicle for intracellular transport of botulinum neurotoxin antagonists. *FEBS Letters* 513, 163–168.

- Guo, J., Wang, J., Gao, S., Ji, B., Waichi Chan, E., & Chen, S. (2015). Substrate-based inhibitors exhibiting excellent protective and therapeutic effects against Botulinum Neurotoxin A intoxication. *Scientific Reports* 5, 16981. <https://doi.org/10.1038/srep16981>.
- Gutman, G. A., Chandy, K. G., Grissmer, S., Lazdunski, M., McKinnon, D., Pardo, L. A., et al. (2005). International union of pharmacology. LIII. Nomenclature and molecular relationships of voltage-gated potassium channels. *Pharmacological Reviews* 57, 473–508. <https://doi.org/10.1124/pr.57.4.10>.
- Halliwel, J. V., & Dolly, J. O. (1982a). Electrophysiological analysis of the presynaptic action of beta-bungarotoxin in the central nervous system. *Toxicon: Official Journal of the International Society on Toxinology* 20, 121–127.
- Halliwel, J. V., & Dolly, J. O. (1982b). Preferential action of beta-bungarotoxin at nerve terminal regions in the hippocampus. *Neuroscience Letters* 30, 321–327.
- Halliwel, J. V., Othman, I. B., Pelchen-Matthews, A., & Dolly, J. O. (1986). Central action of dendrotoxin: Selective reduction of a transient K conductance in hippocampus and binding to localized receptors. *Proceedings of the National Academy of Sciences of the United States of America* 83, 493–497.
- Hart, I. K., Maddison, P., Newsom-Davis, J., Vincent, A., & Mills, K. R. (2002). Phenotypic variants of autoimmune peripheral nerve hyperexcitability. *Brain: A Journal of Neurology* 125, 1887–1895.
- Harvey, A. L. (2001). Twenty years of dendrotoxins. *Toxicon: Official Journal of the International Society on Toxinology* 39, 15–26.
- Harvey, A. L. (2014). Toxins and drug discovery. *Toxicon: Official Journal of the International Society on Toxinology* 92, 193–200. <https://doi.org/10.1016/j.toxicol.2014.10.020>.
- Harvey, A. L., & Karlsson, E. (1982). Protease inhibitor homologues from mamba venoms: Facilitation of acetylcholine release and interactions with prejunctional blocking toxins. *British Journal of Pharmacology* 77, 153–161.
- Hiramatsu, H., Tadokoro, S., Nakanishi, M., & Hirashima, N. (2010). Latrotoxin-induced exocytosis in mast cells transfected with latrophilin. *Toxicon: Official Journal of the International Society on Toxinology* 56, 1372–1380. <https://doi.org/10.1016/j.toxicol.2010.08.002>.
- Hmed, B., Serria, H. T., & Mounir, Z. K. (2013). Scorpion peptides: Potential use for new drug development. *Journal of Toxicology* 2013, 958797. <https://doi.org/10.1155/2013/958797>.
- Holz, G. G., & Habener, J. F. (1998). Black widow spider alpha-latrotoxin: A presynaptic neurotoxin that shares structural homology with the glucagon-like peptide-1 family of insulin secretagogic hormones. *Comparative Biochemistry & Physiology B* 121, 177–184.
- Humeau, Y., Doussau, F., Grant, N. J., & Poulain, B. (2000). How botulinum and tetanus neurotoxins block neurotransmitter release. *Biochimie* 82, 427–446.
- Imredy, J. P., & MacKinnon, R. (2000). Energetic and structural interactions between delta-dendrotoxin and a voltage-gated potassium channel. *Journal of Molecular Biology* 296, 1283–1294. <https://doi.org/10.1006/jmbi.2000.3522>.
- Inagaki, A., Frank, C. A., Usachev, Y. M., Benveniste, M., & Lee, A. (2014). Pharmacological correction of gating defects in the voltage-gated Ca_v2.1 Ca²⁺(+) channel due to a familial hemiplegic migraine mutation. *Neuron* 81, 91–102.
- Jacobs, B. C., Koga, M., van Rijs, W., Geleijns, K., van Doorn, P. A., Willison, H. J., et al. (2008). Subclass IgG to motor gangliosides related to infection and clinical course in Guillain-Barre syndrome. *Journal of Neuroimmunology* 194, 181–190. <https://doi.org/10.1016/j.jneuroim.2007.11.017>.
- Jahn, R., & Scheller, R. H. (2006). SNAREs—Engines for membrane fusion. *Nature Reviews Molecular Cell Biology* 7, 631–643. <https://doi.org/10.1038/nrm2002>.
- Jankovic, J. (2004). Botulinum toxin in clinical practice. *Journal of Neurology, Neurosurgery, and Psychiatry* 75, 951–957.
- Jankovic, J. (2009). Treatment of hyperkinetic movement disorders. *The Lancet Neurology* 8, 844–856. [https://doi.org/10.1016/S1474-4422\(09\)70183-8](https://doi.org/10.1016/S1474-4422(09)70183-8).
- Jenkins, C. N., & Van Houtan, K. S. (2016). Global and regional priorities for marine biodiversity protection. *Biological Conservation* 204, 333–339. <https://doi.org/10.1016/j.biocon.2016.10.005>.
- Jimenez-Tenorio, M., & Tucker, J. K. (2009). *Systematic Classification of Recent and Fossil Conoidean Gastropods: With Keys to the Genera of Cone Shells*. ConchBooks.
- Kaczorowski, G. J., McManus, O. B., Priest, B. T., & Garcia, M. L. (2008). Ion channels as drug targets: The next GPCRs. *The Journal of General Physiology* 131, 399–405. <https://doi.org/10.1085/jgp.200709946>.
- King, G. F. (2011). Venoms as a platform for human drugs: Translating toxins into therapeutics. *Expert Opinion on Biological Therapy* 11, 1469–1484. <https://doi.org/10.1517/14712598.2011.621940>.
- Kiris, E., Burnett, J. C., Kane, C. D., & Bavari, S. (2014). Recent advances in botulinum neurotoxin inhibitor development. *Current Topics in Medicinal Chemistry* 14, 2044–2061.
- Knight, A., Carvajal, J., Schneider, H., Coutelle, C., Chamberlain, S., & Fairweather, N. (1999). Non-viral neuronal gene delivery mediated by the HC fragment of tetanus toxin. *European Journal of Biochemistry* 259, 762–769.
- Koh, D. C., Armugam, A., & Jeyaseelan, K. (2006). Snake venom components and their applications in biomedicine. *Cellular and Molecular Life Sciences* 63, 3030–3041. <https://doi.org/10.1007/s00018-006-6315-0>.
- Kondo, K., Narita, K., & Lee, C. Y. (1978). Amino acid sequences of the two polypeptide chains in beta1-bungarotoxin from the venom of Bungarus multicinctus. *Journal of Biochemistry* 83, 101–115.
- Kondo, K., Toda, H., & Narita, K. (1978). Characterization of phospholipase A activity of beta1-bungarotoxin from Bungarus multicinctus venom. II. Identification of the histidine residue of beta1-bungarotoxin modified by p-bromophenacyl bromide. *Journal of Biochemistry* 84, 1301–1308.
- Kondo, T., Ikenaka, K., Fujimoto, I., Aimoto, S., Kato, H., Ito, K., et al. (1992). K⁺ channel involvement in induction of synaptic enhancement by mast cell degranulating (MCD) peptide. *Neuroscience Research* 13, 207–216.
- Krasnoperov, V., Bittner, M. A., Mo, W. J., Buryanovsky, L., Neubert, T. A., Holz, R. W., et al. (2002). Protein-tyrosine phosphatase-sigma is a novel member of the functional family of alpha-latrotoxin receptors. *Journal of Biological Chemistry* 277, 35887–35895. <https://doi.org/10.1074/jbc.M205478200>.
- Kullmann, F. A., de Groat, W. C., & Artim, D. E. (2009). *Bungarotoxins*. Philadelphia, Pa, London: Saunders, Elsevier.
- Kunkler, P. E., & Kraig, R. P. (2004). P/Q Ca²⁺ channel blockade stops spreading depression and related pyramidal neuronal Ca²⁺ rise in hippocampal organ culture. *Hippocampus* 14, 356–367. <https://doi.org/10.1002/hipo.10181>.
- Kusunoki, S., Kaida, K., & Ueda, M. (2008). Antibodies against gangliosides and ganglioside complexes in Guillain-Barre syndrome: New aspects of research. *Biochimica et Biophysica Acta* 1780, 441–444. <https://doi.org/10.1016/j.bbagen.2007.10.001>.
- Kwong, P. D., McDonald, N. Q., Sigler, P. B., & Hendrickson, W. A. (1995). Structure of beta 2-bungarotoxin: Potassium channel binding by Kunitz modules and targeted phospholipase action. *Structure* 3, 1109–1119.
- Lacy, D. B., & Stevens, R. C. (1997). Recombinant expression and purification of the botulinum neurotoxin type A translocation domain. *Protein Expression and Purification* 11, 195–200. <https://doi.org/10.1006/prep.1997.0772>.
- Lacy, D. B., Tepp, W., Cohen, A. C., DasGupta, B. R., & Stevens, R. C. (1998). Crystal structure of botulinum neurotoxin type A and implications for toxicity. *Nature Structural Biology* 5, 898–902. <https://doi.org/10.1038/23338>.
- Lalli, G., Bohnert, S., Deinhardt, K., Verastegui, C., & Schiavo, G. (2003). The journey of tetanus and botulinum neurotoxins in neurons. *Trends in Microbiology* 11, 431–437.
- Lang, B., & Vincent, A. (2009). Autoimmune disorders of the neuromuscular junction. *Current Opinion in Pharmacology* 9, 336–340. <https://doi.org/10.1016/j.coph.2009.04.005>.
- Layer, R. T., & McIntosh, J. M. (2006). Conotoxins: Therapeutic potential and application. *Marine Drugs* 4, 119–142.
- Lee, S., Kim, Y., Back, S. K., Choi, H. W., Lee, J. Y., Jung, H. H., et al. (2010). Analgesic effect of highly reversible omega-conotoxin FVIA on N type Ca²⁺ channels. *Molecular Pain* 6, 97. <https://doi.org/10.1186/1744-8069-6-97>.
- Leite, M. I., Jacob, S., Viegas, S., Cossins, J., Clover, L., Morgan, B. P., et al. (2008). IgG1 antibodies to acetylcholine receptors in 'seronegative' myasthenia gravis. *Brain: A Journal of Neurology* 131, 1940–1952. <https://doi.org/10.1093/brain/awn092>.
- Lewis, R. J., Dutertre, S., Vetter, I., & Christie, M. J. (2012). Conus venom peptide pharmacology. *Pharmacological Reviews* 64, 259–298. <https://doi.org/10.1124/pr.111.005322>.
- Lewis, R. J., & Garcia, M. L. (2003). Therapeutic potential of venom peptides. *Nature Reviews Drug Discovery* 2, 790–802. <https://doi.org/10.1038/nrd1197>.
- Lewis, R. L., & Gutmann, L. (2004). Snake venoms and the neuromuscular junction. *Seminars in Neurology* 24, 175–179. <https://doi.org/10.1055/s-2004-830904>.
- Li, Y., Foran, P., Lawrence, G., Mohammed, N., Chan-Kwo-Chion, C. K., Lisk, G., et al. (2001). Recombinant forms of tetanus toxin engineered for examining and exploiting neuronal trafficking pathways. *The Journal of Biological Chemistry* 276, 31394–31401. <https://doi.org/10.1074/jbc.M103517200>.
- Linial, M., Ilouz, N., & Feinstein, N. (1995). alpha-latrotoxin is a potent inducer of neurotransmitter release in Torpedo electric organ—Functional and morphological characterization. *The European Journal of Neuroscience* 7, 742–752.
- Livett, B. G., Gayler, K. R., & Khalil, Z. (2004). Drugs from the sea: Conopeptides as potential therapeutics. *Current Medicinal Chemistry* 11, 1715–1723.
- Llinas, R., Sugimori, M., Hillman, D. E., & Cherksey, B. (1992). Distribution and functional significance of the P-type, voltage-dependent Ca²⁺ channels in the mammalian central nervous system. *Trends in Neurosciences* 15, 351–355.
- Llinas, R. R. (2014). Intrinsic electrical properties of mammalian neurons and CNS function: A historical perspective. *Frontiers in Cellular Neuroscience* 8, 320. <https://doi.org/10.3389/fncel.2014.00320>.
- Logonder, U., Jenko-Praznikar, Z., Scott-Davey, T., Pungercar, J., Krizaj, I., & Harris, J. B. (2009). Ultrastructural evidence for the uptake of a neurotoxic snake venom phospholipase A2 into mammalian motor nerve terminals. *Experimental Neurology* 219, 591–594. <https://doi.org/10.1016/j.expneurol.2009.07.017>.
- Maddison, P., Newsom-Davis, J., Mills, K. R., & Souhami, R. L. (1999). Favourable prognosis in Lambert-Eaton myasthenic syndrome and small-cell lung carcinoma. *Lancet* 353, 117–118. [https://doi.org/10.1016/S0140-6736\(05\)76153-5](https://doi.org/10.1016/S0140-6736(05)76153-5).
- Madsen, J. M. (2001). Toxins as weapons of mass destruction. A comparison and contrast with biological-warfare and chemical-warfare agents. *Clinics in Laboratory Medicine* 21, 593–605.
- Malmberg, A. B., & Yaksh, T. L. (1994). Antinociception produced by spinal delivery of the S and R enantiomers of flurbiprofen in the formalin test. *European Journal of Pharmacology* 256, 205–209.
- Matteoli, M., Haimann, C., Torri-Tarelli, F., Polak, J. M., Ceccarelli, B., & De Camilli, P. (1988). Differential effect of alpha-latrotoxin on exocytosis from small synaptic vesicles and from large dense-core vesicles containing calcitonin gene-related peptide at the frog neuromuscular junction. *Proceedings of the National Academy of Sciences of the United States of America* 85, 7366–7370.
- Matthews, C. C., Fishman, P. S., & Wittenberg, G. F. (2014). Tetanus toxin reduces local and descending regulation of the H-reflex. *Muscle & Nerve* 49, 495–501.
- McAlexander, M. A., & Undem, B. J. (2000). Potassium channel blockade induces action potential generation in guinea-pig airway vagal afferent neurones. *Journal of the Autonomic Nervous System* 78, 158–164.
- McDonough, S. I., Boland, L. M., Mintz, I. M., & Bean, B. P. (2002). Interactions among toxins that inhibit N-type and P-type calcium channels. *The Journal of General Physiology* 119, 313–328.
- McMahon, H. T., Foran, P., Dolly, J. O., Verhage, M., Wiegand, V. M., & Nicholls, D. G. (1992). Tetanus toxin and botulinum toxins type A and B inhibit glutamate, gamma-aminobutyric acid, aspartate, and met-enkephalin release from synaptosomes. Clues to the locus of action. *The Journal of Biological Chemistry* 267, 21338–21343.

- McMahon, H. T., Rosenthal, L., Meldolesi, J., & Nicholls, D. G. (1990). Alpha-latrotoxin releases both vesicular and cytoplasmic glutamate from isolated nerve terminals. *Journal of Neurochemistry* 55, 2039–2047.
- Meir, A., Ginsburg, S., Butkevich, A., Kachalsky, S. G., Kaiserman, I., Ahdut, R., et al. (1999). Ion channels in presynaptic nerve terminals and control of transmitter release. *Physiological Reviews* 79, 1019–1088. <https://doi.org/10.1152/physrev.1999.79.3.1019>.
- Meldolesi, J., Madeddu, L., Torda, M., Gatti, G., & Niutta, E. (1983). The effect of alpha-latrotoxin on the neurosecretory PC12 cell line: Studies on toxin binding and stimulation of transmitter release. *Neuroscience* 10, 997–1009.
- Meng, J., Ovsepián, S. V., Wang, J., Pickering, M., Sasse, A., Aoki, K. R., et al. (2009). Activation of TRPV1 mediates calcitonin gene-related peptide release, which excites trigeminal sensory neurons and is attenuated by a retargeted botulinum toxin with antinociceptive potential. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 29, 4981–4992. <https://doi.org/10.1523/JNEUROSCI.5490-08.2009>.
- Mesngon, M., & McNutt, P. (2011). Alpha-latrotoxin rescues SNAP-25 from BoNT/A-mediated proteolysis in embryonic stem cell-derived neurons. *Toxins* 3, 489–503. <https://doi.org/10.3390/toxins3050489>.
- Meunier, F. A., Schiavo, G., & Molgo, J. (2002). Botulinum neurotoxins: From paralysis to recovery of functional neuromuscular transmission. *Journal of Physiology, Paris* 96, 105–113.
- Mintz, I. M., Venema, V. J., Swiderek, K. M., Lee, T. D., Bean, B. P., & Adams, M. E. (1992). P-type calcium channels blocked by the spider toxin omega-Aga-IVA. *Nature* 355, 827–829. <https://doi.org/10.1038/355827a0>.
- Montal, M. (2010). Botulinum neurotoxin: A marvel of protein design. *Annual Review of Biochemistry* 79, 591–617. <https://doi.org/10.1146/annurev.biochem.051908.125345>.
- Montecucco, C. (1986). How do tetanus and botulinum toxins bind to neuronal membranes? *Trends in Biochemical Sciences* 11, 314–317.
- Montecucco, C., & Rossetto, O. (2000). How do presynaptic PLA2 neurotoxins block nerve terminals? *Trends in Biochemical Sciences* 25, 266–270.
- Montecucco, C., & Schiavo, G. (1994). Mechanism of action of tetanus and botulinum neurotoxins. *Molecular Microbiology* 13, 1–8.
- Mulle, C., & Changeux, J. P. (1990). A novel type of nicotinic receptor in the rat central nervous system characterized by patch-clamp techniques. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 10, 169–175.
- Nashmi, R., & Fehlings, M. G. (2001). Mechanisms of axonal dysfunction after spinal cord injury: With an emphasis on the role of voltage-gated potassium channels. *Brain Research Reviews* 38, 165–191.
- Nebe, J., Vanegas, H., Neugebauer, V., & Schaible, H. G. (1997). Omega-agatoxin IVA, a P-type calcium channel antagonist, reduces nociceptive processing in spinal cord neurons with input from the inflamed but not from the normal knee joint—An electrophysiological study in the rat in vivo. *The European Journal of Neuroscience* 9, 2193–2201.
- Negro, S., Lessi, S., Duregotti, E., Aretini, P., La Ferla, M., et al. (2017). CXCL12 α /SDF-1 from perisynaptic Schwann cells promotes regeneration of injured motor axon terminals. *EMBO Molecular Medicine* 9(8), 1000–1010.
- Nicholls, D., Snelling, R., & Dolly, J. O. (1985). Bioenergetic actions of beta-bungarotoxin, dendrotoxin and bee-venom phospholipase A2 on guinea-pig synaptosomes. *The Biochemical Journal* 229, 653–662.
- Nimmrich, V., & Gross, G. (2012). P/Q-type calcium channel modulators. *British Journal of Pharmacology* 167, 741–759. <https://doi.org/10.1111/j.1476-5381.2012.02069.x>.
- Oh, H. M., & Chung, M. E. (2015). Botulinum toxin for neuropathic pain: A review of the literature. *Toxins* 7, 3127–3154. <https://doi.org/10.3390/toxins7083127>.
- O'Leary, V. B., Ovsepián, S. V., Bodeker, M., & Dolly, J. O. (2013). Improved lentiviral transduction of ALS motoneurons in vivo via dual targeting. *Molecular Pharmacology* 10, 4195–4206. <https://doi.org/10.1021/mp400247t>.
- O'Leary, V. B., Ovsepián, S. V., Raghunath, A., Huo, Q., Lawrence, G. W., Smith, L., et al. (2011). Innocuous full-length botulinum neurotoxin targets and promotes the expression of lentiviral vectors in central and autonomic neurons. *Gene Therapy* 18, 656–665. <https://doi.org/10.1038/gt.2011.8>.
- Olivera, B. M., Gray, W. R., Zeikus, R., McIntosh, J. M., Varga, J., Rivier, J., et al. (1985). Peptide neurotoxins from fish-hunting cone snails. *Science* 230, 1338–1343.
- Olivera, B. M., Seger, J., Horvath, M. P., & Fedosov, A. E. (2015). Prey-capture strategies of fish-hunting cone snails: Behavior. *Neurobiology and Evolution* 86, 58–74. <https://doi.org/10.1159/000438449>.
- Orlova, E. V., Rahman, M. A., Gowen, B., Volynski, K. E., Ashton, A. C., Manser, C., et al. (2000). Structure of alpha-latrotoxin oligomers reveals that divalent cation-dependent tetramers form membrane pores. *Nature Structural Biology* 7, 48–53. <https://doi.org/10.1038/71247>.
- Ovsepián, S. V., Bodeker, M., O'Leary, V. B., Lawrence, G. W., & Oliver, D. J. (2015). Internalization and retrograde axonal trafficking of tetanus toxin in motor neurons and transsynaptic propagation at central synapses exceed those of its C-terminal-binding fragments. *Brain Structure & Function* 220, 1825–1838. <https://doi.org/10.1007/s00429-015-1004-0>.
- Ovsepián, S. V., & Dolly, J. O. (2011). Dendritic SNAREs add a new twist to the old neuron theory. *Proceedings of the National Academy of Sciences of the United States of America* 108, 19113–19120. <https://doi.org/10.1073/pnas.1071235108>.
- Ovsepián, S. V., & Friel, D. D. (2008). The leaner P/Q-type calcium channel mutation renders cerebellar Purkinje neurons hyper-excitable and eliminates Ca²⁺-Na⁺ spike bursts. *The European Journal of Neuroscience* 27, 93–103. <https://doi.org/10.1111/j.1460-9568.2007.05998.x>.
- Ovsepián, S. V., LeBerre, M., Steuber, V., O'Leary, V. B., Leibold, C., & Oliver, D. J. (2016). Distinctive role of KV1.1 subunit in the biology and functions of low threshold K(+) channels with implications for neurological disease. *Pharmacology & Therapeutics* 159, 93–101. <https://doi.org/10.1016/j.pharmthera.2016.01.005>.
- Ovsepián, S. V., O'Leary, V. B., Ntziachristos, V., & Dolly, J. O. (2016). Circumventing brain barriers: nanovehicles for retroaxonal therapeutic delivery. *Trends in Molecular Medicine* 22, 983–993. <https://doi.org/10.1016/j.molmed.2016.09.004>.
- Ovsepián, S. V., Steuber, V., LeBerre, M., O'Hara, L., O'Leary, V. B., & Dolly, J. O. (2013). A defined heteromeric KV1 channel stabilizes the intrinsic pacemaking and regulates the output of deep cerebellar nuclear neurons to thalamic targets. *The Journal of Physiology* 591, 1771–1791. <https://doi.org/10.1113/jphysiol.2012.249706>.
- de Paiva, A., Ashton, A. C., Foran, P., Schiavo, G., Montecucco, C., & Dolly, J. O. (1993). Botulinum A like type B and tetanus toxin fulfills criteria for being a zinc-dependent protease. *Journal of Neurochemistry* 61, 2338–2341.
- de Paiva, A., Meunier, F. A., Molgo, J., Aoki, K. R., & Dolly, J. O. (1999). Functional repair of motor endplates after botulinum neurotoxin type A poisoning: Biphasic switch of synaptic activity between nerve sprouts and their parent terminals. *Proceedings of the National Academy of Sciences of the United States of America* 96, 3200–3205.
- Paracelsus, T. (1538). *Die dritte Defension wegen des Schreibens der neuen Rezepte*. Darmstadt.
- Parcej, D. N., & Dolly, J. O. (1989). Dendrotoxin receptor from bovine synaptic plasma membranes. Binding properties, purification and subunit composition of a putative constituent of certain voltage-activated K⁺ channels. *The Biochemical Journal* 257, 899–903.
- Park, J., & Luo, Z. D. (2010). Calcium channel functions in pain processing. *Channels* 4, 510–517.
- Parks, T. N., Artman, L. D., Alasti, N., & Nemeth, E. F. (1991). Modulation of N-methyl-D-aspartate receptor-mediated increases in cytosolic calcium in cultured rat cerebellar granule cells. *Brain Research* 552, 13–22.
- Patel, R., Montagut-Bordas, C., & Dickenson, A. H. (2017). Calcium channel modulation as a target in chronic pain control. *British Journal of Pharmacology*. <https://doi.org/10.1111/bph.13789>.
- Peigneur, S., & Tytgat, J. (2018). Toxins in drug discovery and pharmacology. *Toxins* 10. <https://doi.org/10.3390/toxins10030126>.
- Pellkofer, H. L., Armbruster, L., Krumbholz, M., Titulaer, M. J., Verschuuren, J. J., Schumm, F., et al. (2008). Lambert-eaton myasthenic syndrome differential reactivity of tumor versus non-tumor patients to subunits of the voltage-gated calcium channel. *Journal of Neuroimmunology* 204, 136–139. <https://doi.org/10.1016/j.jneuroim.2008.08.002>.
- Perry, T., & Greig, N. H. (2003). The glucagon-like peptides: A double-edged therapeutic sword? *Trends in Pharmacological Sciences* 24, 377–383. [https://doi.org/10.1016/S0165-6147\(03\)00160-3](https://doi.org/10.1016/S0165-6147(03)00160-3).
- Pirazzini, M., Azarnia Tehran, D., Leka, O., Zanetti, G., Rossetto, O., & Montecucco, C. (2016). On the translocation of botulinum and tetanus neurotoxins across the membrane of acidic intracellular compartments. *Biochimica et Biophysica Acta* 1858, 467–474. <https://doi.org/10.1016/j.bbame.2015.08.014>.
- Pirazzini, M., Azarnia Tehran, D., Zanetti, G., Megighian, A., Scorsetto, M., Fillo, S., et al. (2014). Thioredoxin and its reductase are present on synaptic vesicles, and their inhibition prevents the paralysis induced by botulinum neurotoxins. *Cell Reports* 8, 1870–1878. <https://doi.org/10.1016/j.celrep.2014.08.017>.
- Pirazzini, M., Rossetto, O., Eleopra, R., & Montecucco, C. (2017). Botulinum neurotoxins: Biology, pharmacology, and toxicology. *Pharmacological Reviews* 69, 200–235. <https://doi.org/10.1124/pr.116.012658>.
- Poulter, M. O., Hashiguchi, T., & Padjen, A. L. (1989). Dendrotoxin blocks accommodation in frog myelinated axons. *Journal of Neurophysiology* 62, 174–184. <https://doi.org/10.1152/jn.1989.62.1.174>.
- Prikhodko, G. G., Robson, M., Warmke, J. W., Cohen, C. J., Smith, M. M., Wang, P. Y., et al. (1996). Properties of three baculovirus-expressing genes that encode insect-selective toxins: mu-Aga-IV, As II, and SH I. *Biological Control* 7, 236–244.
- Pringos, E., Vignes, M., Martinez, J., & Rolland, V. (2011). Peptide neurotoxins that affect voltage-gated calcium channels: A close-up on omega-agatoxins. *Toxins* 3, 17–42. <https://doi.org/10.3390/toxins3010017>.
- Qerama, E., Fuglsang-Frederiksen, A., & Jensen, T. S. (2010). The role of botulinum toxin in management of pain: An evidence-based review. *Current Opinion in Anaesthesiology* 23, 602–610. <https://doi.org/10.1097/ACO.0b013e32833c3405>.
- Quistad, G. B., Suwanrumpha, S., Jarema, M. A., Shapiro, M. J., Skinner, W. S., Jamieson, G. C., et al. (1990). Structures of paralytic acylpolyamines from the spider *Agelenopsis aperta*. *Biochemical and Biophysical Research Communications* 169, 51–56.
- Rahman, M. A., Ashton, A. C., Meunier, F. A., Davletov, B. A., Dolly, J. O., & Ushkaryov, Y. A. (1999). Norepinephrine exocytosis stimulated by alpha-latrotoxin requires both external and stored Ca²⁺ and is mediated by latrophilin, G proteins and phospholipase C. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences* 354, 379–386. <https://doi.org/10.1098/rstb.1999.0390>.
- Ramirez, D., Gonzalez, W., Fissore, R. A., & Carvacho, I. (2017). Conotoxins as tools to understand the physiological function of voltage-gated calcium (Ca-V) channels. *Marine Drugs* 15. <https://doi.org/10.3390/Md15100313>.
- Randall, A. D., & Tsien, R. W. (1997). Contrasting biophysical and pharmacological properties of T-type and R-type calcium channels. *Neuropharmacology* 36, 879–893.
- Rigo, F. K., Dalmolin, G. D., Trevisan, G., Tonello, R., Silva, M. A., Rossato, M. F., et al. (2013). Effect of omega-conotoxin MVIIA and Phalpa1beta on paclitaxel-induced acute and chronic pain. *Pharmacology, Biochemistry, and Behavior* 114–115, 16–22.
- Rigo, F. K., Trevisan, G., Rosa, F., Dalmolin, G. D., Otuki, M. F., Cueto, A. P., et al. (2013). Spider peptide Phalpa1beta induces analgesic effect in a model of cancer pain. *Cancer Science* 104, 1226–1230. <https://doi.org/10.1111/cas.12209>.
- Rigoni, M., & Montecucco, C. (2017). Animal models for studying motor axon terminal paralysis and recovery. *Journal of Neurochemistry* 142(Suppl. 2), 122–129. <https://doi.org/10.1111/jnc.13956> (Epub 2017 Mar 21).
- Rigoni, M., Paoli, M., Milanese, E., Caccin, P., Rasola, A., Bernardi, P., et al. (2008). Snake phospholipase A2 neurotoxins enter neurons, bind specifically to mitochondria, and

- open their transition pores. *The Journal of Biological Chemistry* 283, 34013–34020. <https://doi.org/10.1074/jbc.M803243200>.
- Rigoni, M., Schiavo, G., Weston, A. E., Caccin, P., Allegrini, F., Pennuto, M., et al. (2004). Snake presynaptic neurotoxins with phospholipase A2 activity induce punctate swellings of neurites and exocytosis of synaptic vesicles. *Journal of Cell Science* 117, 3561–3570. <https://doi.org/10.1242/jcs.01218>.
- Robbins, C. A., & Tempel, B. L. (2012). Kv1.1 and Kv1.2: Similar channels, different seizure models. *Epilepsia* 53(Suppl. 1), 134–141.
- Robertson, B., Owen, D., Stow, J., Butler, C., & Newland, C. (1996). Novel effects of dendrotoxin homologues on subtypes of mammalian Kv1 potassium channels expressed in *Xenopus* oocytes. *FEBS Letters* 383, 26–30.
- Robinson, S. D., Undheim, E. A. B., Ueberheide, B., & King, G. F. (2017). Venom peptides as therapeutics: Advances, challenges and the future of venom-peptide discovery. *Expert Review of Proteomics* 14, 931–939. <https://doi.org/10.1080/14789450.2017.1377613>.
- Rosenthal, L., Zacchetti, D., Madeddu, L., & Meldolesi, J. (1990). Mode of action of alpha-latrotoxin: Role of divalent cations in Ca²⁺(+)-dependent and Ca²⁺(+)-independent effects mediated by the toxin. *Molecular Pharmacology* 38, 917–923.
- Ross, B. M., Moszczynska, A., Erlich, J., & Kish, S. J. (1998). Phospholipid-metabolizing enzymes in Alzheimer's disease: Increased lysophospholipid acyltransferase activity and decreased phospholipase A2 activity. *Journal of Neurochemistry* 70, 786–793.
- Rossetto, O., Seveso, M., Caccin, P., Schiavo, G., & Montecucco, C. (2001). Tetanus and botulinum neurotoxins: Turning bad guys into good by research. *Toxicom: Official Journal of the International Society on Toxicology* 39, 27–41.
- Ryan, N. M., Buckley, N. A., & Graudins, A. (2017). Treatments for latrodectism-A systematic review on their clinical effectiveness. *Toxins* 9. <https://doi.org/10.3390/toxins9040148>.
- Ryu, J. H., Jung, H. J., Konishi, S., Kim, H. H., Park, Z. Y., & Kim, J. I. (2017). Structure-activity relationships of omega-Agatoxin IVA in lipid membranes. *Biochemical and Biophysical Research Communications* 482, 170–175. <https://doi.org/10.1016/j.bbrc.2016.11.025>.
- Saez, N. J., Senff, S., Jensen, J. E., Er, S. Y., Herzig, V., Rash, L. D., et al. (2010). Spider-venom peptides as therapeutics. *Toxins* 2, 2851–2871. <https://doi.org/10.3390/toxins2122851>.
- Sakaba, T., Stein, A., Jahn, R., & Neher, E. (2005). Distinct kinetic changes in neurotransmitter release after SNARE protein cleavage. *Science* 309, 491–494. <https://doi.org/10.1126/science.1112645>.
- Sanford, M. (2013). Intrathecal ziconotide: A review of its use in patients with chronic pain refractory to other systemic or intrathecal analgesics. *CNS Drugs* 27, 989–1002. <https://doi.org/10.1007/s40263-013-0107-5>.
- Sasse, A., Conduit, R., Ryan, D., Woods, W., & Tucker, A. P. (2005). A pharmacotherapy for obstructive sleep apnea. *Sleep* 28, 1015–1016.
- Schiavo, G., Benfenati, F., Poulain, B., Rossetto, O., Polverino de Lauro, P., DasGupta, B. R., et al. (1992). Tetanus and botulinum-B neurotoxins block neurotransmitter release by proteolytic cleavage of synaptobrevin. *Nature* 359, 832–835. <https://doi.org/10.1038/359832a0>.
- Schiavo, G., Ferrari, G., Rossetto, O., & Montecucco, C. (1991). Tetanus toxin receptor. Specific cross-linking of tetanus toxin to a protein of NGF-differentiated PC 12 cells. *FEBS Letters* 290, 227–230.
- Schiavo, G., Poulain, B., Rossetto, O., Benfenati, F., Tauc, L., & Montecucco, C. (1992). Tetanus toxin is a zinc protein and its inhibition of neurotransmitter release and protease activity depend on zinc. *The EMBO Journal* 11, 3577–3583.
- Schiavo, G., Rossetto, O., Catsicas, S., Polverino de Lauro, P., DasGupta, B. R., Benfenati, F., et al. (1993). Identification of the nerve terminal targets of botulinum neurotoxin serotypes A, D, and E. *The Journal of Biological Chemistry* 268, 23784–23787.
- Schwab, M. E., & Thoenen, H. (1976). Electron microscopic evidence for a transsynaptic migration of tetanus toxin in spinal cord motoneurons: An autoradiographic and morphometric study. *Brain Research* 105, 213–227.
- Schweitz, H., Bruhn, T., Guillemare, E., Moinier, D., Lancelin, J. M., Beress, L., et al. (1995). Kalcidulines and kaliseptine. Two different classes of sea anemone toxins for voltage sensitive K⁺ channels. *The Journal of Biological Chemistry* 270, 25121–25126.
- Scott, V. E., Muniz, Z. M., Sewing, S., Lichtinghagen, R., Parcej, D. N., Pongs, O., et al. (1994). Antibodies specific for distinct Kv subunits unveil a heterooligomeric basis for subtypes of alpha-dendrotoxin-sensitive K⁺ channels in bovine brain. *Biochemistry* 33, 1617–1623.
- Sen, I., Grantham, P. A., & Cooper, J. R. (1976). Mechanism of action of beta-bungarotoxin on synaptosomal preparations. *Proceedings of the National Academy of Sciences of the United States of America* 73, 2664–2668.
- Serna, N., Sanchez-Garcia, L., Unzueta, U., Diaz, R., Vazquez, E., Mangués, R., et al. (2018). Protein-Based Therapeutic Killing for Cancer Therapies. *Trends in Biotechnology* 36, 318–335. <https://doi.org/10.1016/j.tibtech.2017.11.007>.
- Serova, M., Ghoul, A., Benhadji, K. A., Faivre, S., Le Tourneau, C., Cvitkovic, E., et al. (2008). Effects of protein kinase C modulation by PEP005, a novel ingenol analogate, on mitogen-activated protein kinase and phosphatidylinositol 3-kinase signaling in cancer cells. *Molecular Cancer Therapeutics* 7, 915–922. <https://doi.org/10.1158/1535-7163.MCT-07-2060>.
- Shone, C. C., Hambleton, P., & Melling, J. (1985). Inactivation of Clostridium botulinum type A neurotoxin by trypsin and purification of two tryptic fragments. Proteolytic action near the COOH-terminus of the heavy subunit destroys toxin-binding activity. *European Journal of Biochemistry* 151, 75–82.
- Silva, J. P., Lelianaova, V., Hopkins, C., Volynski, K. E., & Ushkaryov, Y. (2009). Functional cross-interaction of the fragments produced by the cleavage of distinct adhesion G-protein-coupled receptors. *The Journal of Biological Chemistry* 284, 6495–6506. <https://doi.org/10.1074/jbc.M806979200>.
- Silva, J. P., Suckling, J., & Ushkaryov, Y. (2009). Penelope's web: Using alpha-latrotoxin to untangle the mysteries of exocytosis. *Journal of Neurochemistry* 111, 275–290. <https://doi.org/10.1111/j.1471-4159.2009.06329.x>.
- Silva, M. F., Mota, C. M., Miranda Vdos, S., Cunha Ade, O., Silva, M. C., Naves, K. S., et al. (2015). Biological and enzymatic characterization of proteases from crude venom of the ant *odontomachus bauri*. *Toxins* 7, 5114–5128. <https://doi.org/10.3390/toxins7124869>.
- Simpson, L. L. (2004). Identification of the major steps in botulinum toxin action. *Annual Review of Pharmacology and Toxicology* 44, 167–193. <https://doi.org/10.1146/annurev.pharmtox.44.101802.121554>.
- Singh, J. A. (2010). Botulinum toxin therapy for osteoarticular pain: An evidence-based review. *Therapeutic Advances in Musculoskeletal Disease* 2, 105–118. <https://doi.org/10.1177/1759720X09357113>.
- Skeie, G. O., Apostolski, S., Evoli, A., Gilhus, N. E., Hart, I. K., Harms, L., et al. (2006). Guidelines for the treatment of autoimmune neuromuscular transmission disorders. *European Journal of Neurology* 13, 691–699. <https://doi.org/10.1111/j.1468-1331.2006.01476.x>.
- Skinner, W. S., Adams, M. E., Quistad, G. B., Kataoka, H., Cesarin, B. J., Enderlin, F. E., et al. (1989). Purification and characterization of two classes of neurotoxins from the funnel web spider, *Agelenopsis aperta*. *The Journal of Biological Chemistry* 264, 2150–2155.
- Slotta, K. H., & Fraenkel-Conrat, H. (1938). Two Active Proteins from Rattlesnake Venom. *Nature* 142, 213.
- Sluka, K. A. (1997). Blockade of calcium channels can prevent the onset of secondary hyperalgesia and allodynia induced by intradermal injection of capsaicin in rats. *Pain* 71, 157–164.
- Sluka, K. A. (1998). Blockade of N- and P/Q-type calcium channels reduces the secondary heat hyperalgesia induced by acute inflammation. *The Journal of Pharmacology and Experimental Therapeutics* 287, 232–237.
- Soares, M. B., Bellintani, M. C., Ribeiro, I. M., Tomassini, T. C., & Ribeiro dos Santos, R. (2003). Inhibition of macrophage activation and lipopolysaccharide-induced death by seco-steroids purified from *Physalis angulata* L. *European Journal of Pharmacology* 459, 107–112.
- Sobel, J. (2009). Diagnosis and treatment of botulism: A century later, clinical suspicion remains the cornerstone. *Clinical Infectious Diseases* 48, 1674–1675. <https://doi.org/10.1086/599030>.
- Sobel, J. B. (2005). *Clinical Infectious Diseases* 41, 1167–1173.
- Southan, A. P., & Robertson, B. (1998a). Modulation of inhibitory post-synaptic currents (IPSCs) in mouse cerebellar Purkinje and basket cells by snake and scorpion toxin K⁺ channel blockers. *British Journal of Pharmacology* 125, 1375–1381. <https://doi.org/10.1038/sj.bjp.0702218>.
- Southan, A. P., & Robertson, B. (1998b). Patch-clamp recordings from cerebellar basket cell bodies and their presynaptic terminals reveal an asymmetric distribution of voltage-gated potassium channels. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 18, 948–955.
- Staats, P. S., Yearwood, T., Charapata, S. G., Presley, R. W., Wallace, M. S., Byas-Smith, M., et al. (2004). Intrathecal ziconotide in the treatment of refractory pain in patients with cancer or AIDS: A randomized controlled trial. *JAMA* 291, 63–70. <https://doi.org/10.1001/jama.291.1.63>.
- Stansfeld, C. E., Marsh, S. J., Parcej, D. N., Dolly, J. O., & Brown, D. A. (1987). Mast cell degranulating peptide and dendrotoxin selectively inhibit a fast-activating potassium current and bind to common neuronal proteins. *Neuroscience* 23, 893–902.
- Strong, P. N., Goerke, J., Oberg, S. G., & Kelly, R. B. (1976). beta-Bungarotoxin, a pre-synaptic toxin with enzymatic activity. *Proceedings of the National Academy of Sciences of the United States of America* 73, 178–182.
- Strydom, A. J. (1973a). Snake venom toxins. The amino acid sequences of two toxins from *Dendroaspis jamesoni kaimosae* (Jameson's mamba) venom. *Biochimica et Biophysica Acta* 328, 491–509.
- Strydom, D. J. (1973b). Protease inhibitors as snake venom toxins. *Nature: New Biology* 243, 88–89.
- Strydom, D. J. (1973c). Snake venom toxins. Structure-function relationships and phylogenetics. *Comparative Biochemistry and Physiology B, Comparative Biochemistry* 44, 269–281.
- Sudhof, T. C. (2001). alpha-Latrotoxin and its receptors: Neurexins and CIRL/latrophilins. *Annual Review of Neuroscience* 24, 933–962. <https://doi.org/10.1146/annurev.neuro.24.1.933>.
- Sudhof, T. C., & Rizo, J. (2011). Synaptic vesicle exocytosis. *Cold Spring Harbor Perspectives in Biology* 3. <https://doi.org/10.1101/cshperspect.a005637>.
- Sun, G. Y., Shelat, P. B., Jensen, M. B., He, Y., Sun, A. Y., & Simonyi, A. (2010). Phospholipases A2 and inflammatory responses in the central nervous system. *Neuromolecular Medicine* 12, 133–148. <https://doi.org/10.1007/s12017-009-8092-z>.
- Sutton, R. B., Fasshauer, D., Jahn, R., & Brunger, A. T. (1998). Crystal structure of a SNARE complex involved in synaptic exocytosis at 2.4 Å resolution. *Nature* 395, 347–353. <https://doi.org/10.1038/26412>.
- Takano, K., Kirchner, F., Terhaar, P., & Tiebert, B. (1983). Effect of tetanus toxin on the monosynaptic reflex. *Naunyn-Schmiedeberg's Archives of Pharmacology* 323, 217–220.
- Tehran, D. A., & Pirazzini, M. (2018). Novel botulinum neurotoxins: Exploring underneath the iceberg tip. *Toxins* 10. <https://doi.org/10.3390/toxins10050190>.
- Tehran, D. A., Pirazzini, M., Leka, O., Mattarei, A., Lista, F., Binz, T., et al. (2017). Hsp90 is involved in the entry of clostridial neurotoxins into the cytosol of nerve terminals. *Cellular Microbiology* 19.
- Terlau, H., & Olivera, B. M. (2004). Conus venoms: A rich source of novel ion channel-targeted peptides. *Physiological Reviews* 84, 41–68. <https://doi.org/10.1152/physrev.00020.2003>.
- Tibbs, G. R., Dolly, J. O., & Nicholls, D. G. (1989). Dendrotoxin, 4-aminopyridine, and beta-bungarotoxin act at common loci but by two distinct mechanisms to induce Ca²⁺-

- dependent release of glutamate from guinea-pig cerebrocortical synaptosomes. *Journal of Neurochemistry* 52, 201–206.
- Toivonen, J. M., Olivan, S., & Osta, R. (2010). Tetanus toxin C-fragment: The courier and the cure? *Toxins* 2, 2622–2644. <https://doi.org/10.3390/toxins2112622>.
- Tottene, A., Urbani, A., & Pietrobon, D. (2011). Role of different voltage-gated Ca²⁺ channels in cortical spreading depression: Specific requirement of P/Q-type Ca²⁺ channels. *Channels* 5, 110–114.
- Tsang, C. W., Elrick, D. B., & Charlton, M. P. (2000). alpha-Latrotoxin releases calcium in frog motor nerve terminals. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 20, 8685–8692.
- Turton, K., Chaddock, J. A., & Acharya, K. R. (2002). Botulinum and tetanus neurotoxins: Structure, function and therapeutic utility. *Trends in Biochemical Sciences* 27, 552–558.
- Twede, V. D., Miljanich, G., Olivera, B. M., & Bulaj, G. (2009). Neuroprotective and cardioprotective conopeptides: An emerging class of drug leads. *Current Opinion in Drug Discovery & Development* 12, 231–239.
- Tytgat, J., Debont, T., Carmeliet, E., & Daenens, P. (1995). The alpha-dendrotoxin footprint on a mammalian potassium channel. *The Journal of Biological Chemistry* 270, 24776–24781.
- Uchitel, O. D., Protti, D. A., Sanchez, V., Cherksey, B. D., Sugimori, M., & Llinas, R. (1992). P-type voltage-dependent calcium channel mediates presynaptic calcium influx and transmitter release in mammalian synapses. *Proceedings of the National Academy of Sciences of the United States of America* 89, 3330–3333.
- Usherwood, P. N., & Blagbrough, I. S. (1991). Spider toxins affecting glutamate receptors: Polyamines in therapeutic neurochemistry. *Pharmacology & Therapeutics* 52, 245–268.
- Ushkaryov, Y. A., Rohou, A., & Sugita, S. (2008). alpha-Latrotoxin and its receptors. *Handbook of experimental pharmacology* (pp. 171–206). https://doi.org/10.1007/978-3-540-74805-2_7.
- Utkin, Y. N. (2015). Animal venom studies: Current benefits and future developments. *World Journal of Biological Chemistry* 6, 28–33. <https://doi.org/10.4331/wjbc.v6.i2.28>.
- Velluti, J. C., Caputi, A., & Macadar, O. (1987). Limbic epilepsy induced in the rat by dendrotoxin, a polypeptide isolated from the green mamba (*Dendroaspis angusticeps*) venom. *Toxicon: Official Journal of the International Society on Toxinology* 25, 649–657.
- Vetter, I., & Lewis, R. J. (2012). Therapeutic potential of cone snail venom peptides (conopeptides). *Current Topics in Medicinal Chemistry* 12, 1546–1552.
- Vulfius, C. A., Kasheverov, I. E., Kryukova, E. V., Spirova, E. N., Shelukhina, I. V., Starkov, V. G., et al. (2017). Pancreatic and snake venom presynaptically active phospholipases A2 inhibit nicotinic acetylcholine receptors. *PLoS One* 12, e0186206. <https://doi.org/10.1371/journal.pone.0186206>.
- Wang, F. C., Bell, N., Reid, P., Smith, L. A., McIntosh, P., Robertson, B., et al. (1999). Identification of residues in dendrotoxin K responsible for its discrimination between neuronal K⁺ channels containing Kv1.1 and 1.2 alpha subunits. *European Journal of Biochemistry* 263, 222–229.
- Wang, F. C., Parcej, D. N., & Dolly, J. O. (1999). alpha subunit compositions of Kv1.1-containing K⁺ channel subtypes fractionated from rat brain using dendrotoxins. *European Journal of Biochemistry* 263, 230–237.
- Wang, J., Meng, J., Lawrence, G. W., Zurawski, T. H., Sasse, A., Bodeker, M. O., et al. (2008). Novel chimeras of botulinum neurotoxins A and E unveil contributions from the binding, translocation, and protease domains to their functional characteristics. *The Journal of Biological Chemistry* 283, 16993–17002. <https://doi.org/10.1074/jbc.M710442200>.
- Wang, J., Zurawski, T. H., Bodeker, M. O., Meng, J., Boddul, S., Aoki, K. R., et al. (2012). Longer-acting and highly potent chimeric inhibitors of excessive exocytosis created with domains from botulinum neurotoxin A and B. *The Biochemical Journal* 444, 59–67. <https://doi.org/10.1042/BJ20120100>.
- Wang, Y. X., Gao, D., Pettus, M., Phillips, C., & Bowersox, S. S. (2000). Interactions of intrathecally administered ziconotide, a selective blocker of neuronal N-type voltage-sensitive calcium channels, with morphine on nociception in rats. *Pain* 84, 271–281.
- Wang, Y. X., Pettus, M., Gao, D., Phillips, C., & Scott Bowersox, S. (2000). Effects of intrathecal administration of ziconotide, a selective neuronal N-type calcium channel blocker, on mechanical allodynia and heat hyperalgesia in a rat model of postoperative pain. *Pain* 84, 151–158.
- Webster, L. R., Fakata, K. L., Charapata, S., Fisher, R., & Minehart, M. (2008). Open-label, multicenter study of combined intrathecal morphine and ziconotide: Addition of morphine in patients receiving ziconotide for severe chronic pain. *Pain Medicine* 9, 282–290. <https://doi.org/10.1111/j.1526-4637.2007.00356.x>.
- Weller, U., Bernhardt, U., Siemen, D., Dreyer, F., Vogel, W., & Habermann, E. (1985). Electrophysiological and neurobiochemical evidence for the blockade of a potassium channel by dendrotoxin. *Naunyn-Schmiedeberg's Archives of Pharmacology* 330, 77–83.
- Weller, U., Dauzenroth, M. E., Gansel, M., & Dreyer, F. (1991). Cooperative action of the light chain of tetanus toxin and the heavy chain of botulinum toxin type A on the transmitter release of mammalian motor endplates. *Neuroscience Letters* 122, 132–134.
- Weller, U., Taylor, C. F., & Habermann, E. (1986). Quantitative comparison between tetanus toxin, some fragments and toxoid for binding and axonal transport in the rat. *Toxicon: Official Journal of the International Society on Toxinology* 24, 1055–1063.
- White, J., & Weinstein, S. A. (2015). Latrodectism and effectiveness of antivenom. *Annals of Emergency Medicine* 65, 123–124. <https://doi.org/10.1016/j.annemergmed.2014.08.022>.
- Willison, H. J. (2005). The immunobiology of Guillain-Barre syndromes. *Journal of the Peripheral Nervous System* 10, 94–112.
- Willison, H. J., Jacobs, B. C., & van Doorn, P. A. (2016). Guillain-Barre syndrome. *Lancet* 388, 717–727. [https://doi.org/10.1016/S0140-6736\(16\)00339-1](https://doi.org/10.1016/S0140-6736(16)00339-1).
- Wirtz, P. W., Lang, B., Graus, F., van den Maagdenberg, A. M., Saiz, A., de Koning Gans, P. A., et al. (2005). P/Q-type calcium channel antibodies, Lambert-Eaton myasthenic syndrome and survival in small cell lung cancer. *Journal of Neuroimmunology* 164, 161–165. <https://doi.org/10.1016/j.jneuroim.2005.04.001>.
- Wu, J., Yan, Z., Li, Z., Yan, C., Lu, S., Dong, M., et al. (2015). Structure of the voltage-gated calcium channel Cav1.1 complex. *Science* 350, aad2395. <https://doi.org/10.1126/science.aad2395>.
- Yaksh, T. L. (2006). Calcium channels as therapeutic targets in neuropathic pain. *The Journal of Pain* 7, S13–S30.
- Yan, L., & Adams, M. E. (2000). The spider toxin omega-Aga IIIA defines a high affinity site on neuronal high voltage-activated calcium channels. *The Journal of Biological Chemistry* 275, 21309–21316. <https://doi.org/10.1074/jbc.M000212200>.
- Yan, S., & Wang, X. (2015). Recent advances in research on widow spider venoms and toxins. *Toxins* 7, 5055–5067. <https://doi.org/10.3390/toxins7124862>.
- Yu, R., Kompella, S. N., Adams, D. J., Craik, D. J., & Kaas, Q. (2013). Determination of the alpha-conotoxin Vc1.1 binding site on the alpha9alpha10 nicotinic acetylcholine receptor. *Journal of Medicinal Chemistry* 56, 3557–3567. <https://doi.org/10.1021/jm400041h>.
- Zhang, S., Masuyer, G., Zhang, J., Shen, Y., Lundin, D., Henriksson, L., et al. (2017). Identification and characterization of a novel botulinum neurotoxin. *Nature Communications* 8, 14130. <https://doi.org/10.1038/ncomms14130>.
- Zornetta, I., Azarnia Tehran, D., Arrigoni, G., Annibaldi, F., Bano, L., Leka, O., et al. (2016). The first non Clostridial botulinum-like toxin cleaves VAMP within the juxtamembrane domain. *Scientific Reports* 6, 30257. <https://doi.org/10.1038/srep30257>.
- Zornetta, I., Caccin, P., Fernandez, J., Lomonte, B., Gutierrez, J. M., & Montecucco, C. (2012). Envenomations by Bothrops and Crotalus snakes induce the release of mitochondrial alarmins. *PLoS Neglected Tropical Diseases* 6, e1526. <https://doi.org/10.1371/journal.pntd.0001526>.
- Zupunski, V., & Kordis, D. (2016). Strong and widespread action of site-specific positive selection in the snake venom Kunitz/BPTI protein family. *Scientific Reports* 6, 37054. <https://doi.org/10.1038/srep37054>.