Cell Calcium 52 (2012) 234–240

Contents lists available at SciVerse ScienceDirect

Cell Calcium

journal homepage: www.elsevier.com/locate/ceca

Presynaptic neurotoxins: An expanding array of natural and modified molecules

Bazbek Davletov[a,*], Enrico Ferrari[a], Yuri Ushkaryov[b]

[a] Medical Research Council Laboratory of Molecular Biology, Cambridge, UK
[b] Medway School of Pharmacy, University of Kent, Chatham Maritime, UK

ARTICLE INFO

Article history:
Received 15 February 2012
Received in revised form 4 May 2012
Accepted 13 May 2012
Available online 1 June 2012

Keywords:
Botulinum
Clostridial
Neurotoxin
Latrotoxin
Chimera
SNARE proteins
Black widow spider
Exocytosis
Neurotransmitter release
Presynaptic
Calcium

ABSTRACT

The process of neurotransmitter release from nerve terminals is a target for a wide array of presynaptic toxins produced by various species, from humble bacteria to arthropods to vertebrate animals. Unlike other toxins, most presynaptic neurotoxins do not kill cells but simply inhibit or activate synaptic transmission. In this review, we describe two types of presynaptic neurotoxins: clostridial toxins and latrotoxins, which are, respectively, the most potent blockers and stimulators of neurotransmitter release. These toxins have been instrumental in defining presynaptic functions and are now widely used in research and medicine. Here, we would like to analyse the diversity of these toxins and demonstrate how the knowledge of their structures and mechanisms of action can help us to design better tools for research and medical applications. We will look at natural and synthetic variations of these exquisite molecular machines, highlighting recent advances in our understanding of presynaptic toxins and questions that remain to be answered. If we can decipher how a given biomolecule is modified by nature to target different species, we will be able to design new variants that carry only desired characteristics to achieve specific therapeutic, agricultural or research goals. Indeed, a number of research groups have already initiated a quest to harness the power of natural toxins with the aim of making them more specifically targeted and safer for future research and medical applications.

1. Introduction

As their name suggests, presynaptic neurotoxins target presynaptic terminals where they affect the release of neurotransmitters. First, we would like to describe the presynaptic release mechanisms which were deciphered with the help of these presynaptic toxins. Neurotransmitters are stored in synaptic vesicles within the presynaptic terminal, and their release involves calcium-triggered fusion of the vesicular and presynaptic membranes (Fig. 1). Since both membranes are negatively charged, their repulsion can only be overcome by protein-mediated mechanisms. Presynaptic proteins which facilitate priming and fusion are known as SNARE proteins, an acronym for Soluble NSF Attachment protein Receptors, where NSF is an intracellular enzyme called N-ethylmaleimide Sensitive Factor [1]. Initially, one SNARE protein is found on the vesicular membrane (synaptobrevin also known as VAMP, for vesicle-associated membrane protein), whereas two other SNARE proteins – syntaxin and SNAP-25 – reside in the presynaptic membrane [2]. The three SNARE proteins undergo a series of priming events, which allow them to come into close contact between the membranes [3]. In response to Ca^{2+} entry into the presynaptic terminal, SNARE proteins undergo a coiling reaction facilitating membrane interaction and fusion [2] leading to the release of intravesicular neurotransmitter into the extracellular space. After the release event completed, the SNARE proteins are uncoiled by the NSF enzyme [1] and the vesicle is retrieved – endocytosed – for refilling with a new load of neurotransmitter.

As these fine molecular mechanisms, constituting the basis of neuronal communication, were developing in evolution, they also became a target for toxins produced by organisms from all taxa: bacteria, plants, molluscs, spiders, insects and vertebrates. Aimed at disabling the animal nervous system, neurotoxins have co-evolved with it to become sophisticated and powerful 'magic bullets', exquisitely targeting individual molecular components in presynaptic nerve terminals. Their precise targeting of the presynaptic machinery has made neurotoxins the tools of choice for researchers trying to understand neurotransmitter release mechanisms [4]. Depending on their principal activity, presynaptic neurotoxins can be divided into two groups: inhibitory and excitatory, with the majority of neurotoxins falling into the first group. In addition, toxins can be classified by their main functional target: some neurotoxins perturb the mechanisms of vesicle fusion; the others affect cytosolic Ca^{2+} – the trigger for neurotransmitter release.

In this review, we will look at the most polar representatives of the different groups of presynaptic neurotoxins: the strongest inhibitors of vesicle fusion and the most potent activators of

* Corresponding author. Tel.: +44 1223 402009.
E-mail address: bazbek@mrc-lmb.cam.ac.uk (B. Davletov).

0143-4160/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ceca.2012.05.006

exocytotic signals. We will concentrate on those neurotoxins that can be picked apart, remodelled and reassembled again, in an attempt to produce new tools for research and, as we are beginning to find out, for medical purposes. Based on these considerations, we will take a detailed look at two types of presynaptic neurotoxins: the clostridial neurotoxins and the latrotoxins.

2. Prototypic clostridial neurotoxins

Anaerobic *Clostridium* bacteria, commonly found in soil, produce several presynaptic toxins which potently inhibit synaptic release of neurotransmitters [4]. Due to their large size (150 kDa) these toxins cannot pass blood–brain barrier and, therefore, cause mainly peripheral effects. Toxins produced by *Clostridium botulinum* inactivate neuromuscular transmission and cause long-lasting flaccid paralysis, and thus they have become a blockbuster treatment for persistent muscle spasms and removal of wrinkles (the BOTOX medicine) [5,6]. In contrast, tetanus toxin produced by *Clostridium tetani* has the ability to enter the spinal cord to block central inhibitory neurons causing continuous muscle spasms, tetanus [7]. Despite their outwardly dissimilar effects, botulinum and tetanus toxins have similar principal mechanisms of inhibiting presynaptic release of neurotransmitters.

Both botulinum and tetanus toxins block synaptic vesicle fusion by cleaving the coiling parts of the SNARE proteins involved in vesicle fusion [8]. Among the seven known botulinum toxins (A–G), types A and E cleave the SNAP-25 protein, type C cleaves both SNAP-25 and syntaxin, whereas types B, D, F and G cleave vesicular synaptobrevin (Fig. 1). Tetanus toxin also specifically targets synaptobrevin [9]. Since all three SNAREs are obligatory for vesicle fusion, the end result of the clostridial protease action is a long-lasting blockade of neurotransmitter release, which can be restored only upon substitution of the cleaved SNAREs by newly synthesised molecules. The duration of synaptic blockade is remarkably different between the clostridial toxins, ranging from 1–2 weeks to several months [10]. Several factors could contribute to prolonged synaptic blockade: the distance of the presynaptic terminals from the neuronal cell body, variable persistence of clostridial proteases within the neuron, specific sites of the SNARE cleavage impacting on SNARE assembly, coiling or disassembly [11].

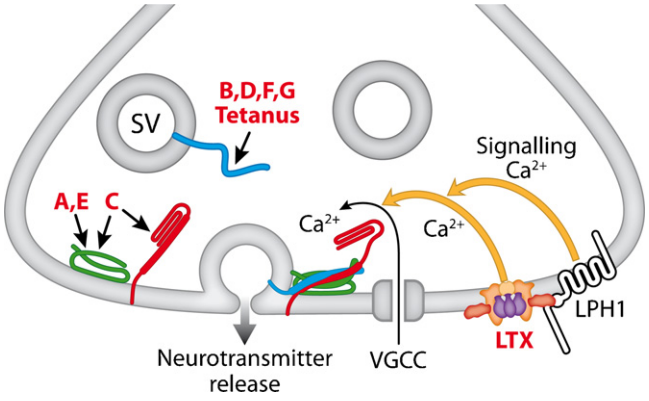

Fig. 1. Presynaptic nerve terminal is the target for clostridial neurotoxins and latrotoxin. Fusion of synaptic vesicles (SVs) with the plasma membrane requires the SNARE proteins: synaptobrevin (blue), SNAP-25 (green) and syntaxin (red). The membrane fusion is triggered by the influx of Ca^{2+} through voltage-gated calcium channels (VGCC). Clostridial neurotoxins of different serotypes (A, B, C, D, E, G, F and tetanus toxin, in red) cleave the SNARE proteins with high specificity and block vesicle fusion. α-Latrotoxin (LTX) binds to its receptors (for example, latrophilin, LPH1) and forms pores in the presynaptic membrane. Vesicle fusion is overstimulated when the terminal is flooded with Ca^{2+} coming from the outside and from intracellular stores.

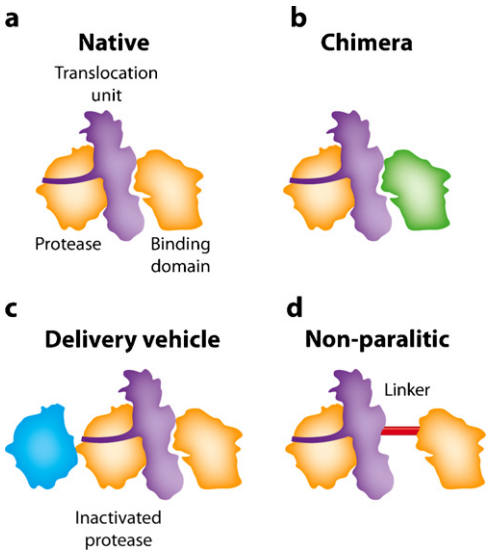

Fig. 2. Natural and modified clostridial toxins. (a) A prototypic clostridial toxin consists of three domains indicated. (b) Natural and synthetic chimeras carry a substituted ganglioside-binding domain (green). (c) Inactivation of clostridial protease allows conversion of the clostridial toxin into a neuron-specific delivery vehicle. The cargo (blue) could be protein, drug or DNA. (d) Structural extension of clostridial toxin allows blocking CNS neurons without affecting neuromuscular junctions.

While the clostridial protease action was well defined in the 1990s (reviewed in [4]), the mode of neuronal targeting and protease translocation into the presynaptic terminals has become clearer only recently. Protein structures have been solved by X-ray crystallography for all clostridial toxins, revealing remarkable structural similarity. The toxins consist of three structurally distinct parts: the protease, the translocation unit and the ganglioside-binding domain (Fig. 2a). The protease is enwrapped by the translocation unit, the two being covalently connected only by a disulphide bond. On the other side of the translocation unit sits the ganglioside-binding domain, together forming so-called heavy chain, a continuous 100 kDa polypeptide chain. Due to its smaller molecular weight (50 kDa) the protease is often called the light chain. Both chains are synthesised by *Clostridium* bacteria as a single 150 kDa protein which is cleaved into the light and heavy chains either by bacterial proteases or by the gut enzymes upon intoxication. Such separation of the light chain is crucial for its eventual entry into the interior of the presynaptic terminal, where the protease cleaves the SNARE proteins. However, prior to reaching the nerve terminals the protease is tightly associated with the translocation unit and is only released after the toxin enters the cytosol, where the reducing environment breaks the disulphide bond. This protease-releasing mechanism is similar among the clostridial toxins [12].

There are certain differences in the way clostridial toxins bind to the nerve terminals and we will first consider the botulinum toxin type C. This toxin enters bloodstream, via epithelial gut cell transcytosis, after ingestion of a bacteria-contaminated food and then binds to peripheral nerves due to its affinity for gangliosides GT1b and GD1a [13]. These gangliosides are normally present on the surface of fast-conducting neurons [14]. Upon binding to the neuronal surface, the botulinum toxin reaches the presynaptic terminal and, during continuous rounds of synaptic vesicle fusion, hijacks a retrieving vesicle. When vesicles are retrieved, their interior undergoes acidification to aid neurotransmitter loading. The botulinum toxin exploits this physiological mechanism since its translocation unit is highly pH-sensitive [15]. Upon acidification, the translocation unit undergoes a conformational change and releases the botulinum protease into the presynaptic cytosol. How