Toxicon 54 (2009) 593–599

Contents lists available at ScienceDirect

Toxicon

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

Central effects of tetanus and botulinum neurotoxins

Matteo Caleo [a,*], Giampietro Schiavo [b,1]

[a] Istituto di Neuroscienze, Consiglio Nazionale delle Ricerche, via G. Moruzzi 1, 56100 Pisa, Italy
[b] Molecular NeuroPathoBiology Laboratory, Cancer Research UK, London Research Institute, 44 Lincoln's Inn Field, London WC2A 3PX, UK

ARTICLE INFO

Article history:
Received 17 September 2008
Received in revised form 4 December 2008
Accepted 8 December 2008
Available online 3 March 2009

Keywords:
Neurotransmitter release
Neuromuscular junction
Motoneurons
Retrograde axonal transport
Neuronal activity
Epilepsy

ABSTRACT

Tetanus neurotoxin (TeNT) and botulinum neurotoxins (BoNTs; from A to G) are metalloproteases that act on nerve terminals to prevent exocytosis. They are extensively exploited for the study of cellular physiology. Moreover, BoNTs are also employed in clinical neurology for the treatment of several disorders characterized by hyperexcitability of peripheral nerve terminals. This review summarizes recent studies that have provided a deeper understanding of the mode of action of TeNT and BoNTs. TeNT and BoNTs bind with extreme specificity and are internalized at the neuromuscular junction. We first examine the retrograde transport mechanisms by which TeNT gains access to the central nervous system. We also discuss recent findings indicating that, besides their well known local actions at the neuromuscular junction, BoNTs can also affect central circuits.

The family of clostridial neurotoxins comprises tetanus neurotoxin (TeNT) and botulinum neurotoxins (BoNTs, from A to G). They are the causative agents of tetanus and botulism, respectively. Tetanus is characterized by spastic paralysis due to a TeNT-mediated blockade of inhibitory circuits in the spinal cord. In contrast, intoxication by BoNT results in flaccid paralysis due to a blockade of acetylcholine (ACh) release at the neuromuscular junction (NMJ). Due to their extreme potency and specificity, TeNT and BoNTs have also been exploited for scientific and therapeutic applications. In experimental settings, they represent valuable tools for the study of cellular physiology. In addition, localized minute injections of BoNTs are increasingly being used in the clinic for the treatment of several human diseases characterized by hyperexcitability of peripheral nerve terminals (Davletov et al., 2005; Montecucco and Molgo, 2005; Naumann et al., 2008; Simpson et al., 2008a,b).

Structurally, TeNT and BoNTs share a common organization, with a heavy (H, 100 kDa) and a light chain (L, 50 kDa) linked by a disulphide bond and non-covalent interactions. The carboxy-terminus of the heavy chain (H_C) binds with extraordinary affinity and specificity to nerve terminals. Following internalization, the amino-terminal portion of the heavy chain (H_N) inserts into the membrane of the endosome at acidic pH and assists the translocation of the L chain into the cytosol. Finally, the L chain is endowed with a zinc-endopeptidase activity specific for SNARE proteins. SNARE proteins are involved in the fusion of synaptic vesicles with the plasma membrane and therefore the catalytic activity of the light chain is to prevent exocytosis and neurotransmission (Jahn and Sudhof, 1999).

Poisoning by TeNT and BoNTs occurs via a sequential mechanism comprising cell binding, internalization, trafficking, translocation into the neuronal cytosol and catalytic cleavage of protein substrates (Meunier et al., 2002; Turton et al., 2002). The neuronal binding domain resides in the C-terminal portion of the heavy chain (H_C). Specifically, there is evidence that the H_C of clostridial neurotoxins binds to both polysialogangliosides and membrane proteins, thus providing support for the "double-receptor model" (Montecucco, 1986). In particular, it has been proposed that TeNT and BoNTs are first captured by an antenna consisting of the oligosaccharide portion of

* Corresponding author. Tel.: +39 50 3153195; fax: +39 50 3153220.
E-mail addresses: caleo@in.cnr.it (M. Caleo), giampietro.schiavo@cancer.org.uk (G. Schiavo).
[1] Tel.: +44 20 72693300; fax: +44 20 72693417.

0041-0101/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.toxicon.2008.12.026

polysialogangliosides. This step yields a membrane concentration that facilitates the subsequent interaction with protein receptor molecules on the plasma membrane (Montecucco et al., 2004). Indeed, it appears that both polysialogangliosides and protein receptors should be present for efficient and productive binding of clostridial toxins to membranes (Dong et al., 2007; Rummel et al., 2007). Interaction with polygangliosides is mediated by an oligosaccharide-binding pocket in the H_C portion of all clostridial toxins (known as the lactose site). TeNT possesses an additional carbohydrate-binding site which has been shown to dock sialic acid (Rummel et al., 2003). Protein receptor molecules have been so far identified only for a subset of clostridial toxins (Dong et al., 2003; Rummel et al., 2004; Dong et al., 2006; Mahrhold et al., 2006). BoNT/B and BoNT/G have been found to bind to synaptotagmins (Syts) I and II, two proteins integral to the synaptic vesicle membrane (Dong et al., 2007; Rummel et al., 2007). The Syt domain recognized by BoNT/B is inside the synaptic vesicle lumen and it is therefore exposed at the synaptic terminal during exocytosis (Schiavo, 2006). This accounts for the well known activity-dependent uptake of BoNTs by synaptic terminals. Another synaptic vesicle protein, SV2, acts as the protein receptor for BoNT/A (Dong et al., 2006; Mahrhold et al., 2006). Again, binding of BoNT/A occurs within a lumenal loop of SV2, accounting for accelerated uptake of the toxin following nerve stimulation (Hughes and Whaler, 1962; Keller et al., 2004; Dong et al., 2006). In spite of these recent discoveries, many questions about the membrane binding of BoNTs remain unanswered. In addition to the lack of functional information on the receptors of BoNT/E and F, protein receptors do not seem to play a major role in the binding of BoNT/C and D (Tsukamoto et al., 2005). Furthermore, recent data indicate that BoNTs may interact with more than one single protein ligand. Indeed, BoNT/A and BoNT/B appear to associate with synaptic vesicle protein complexes comprising SV2, synaptotagmin, synaptophysin, VAMP-2, and several subunits of the vesicular ATPase (Baldwin and Barbieri, 2007). There is also evidence that a growth factor receptor, the fibroblast growth factor (FGF) receptor 3 can bind BoNT/A (Fernandez-Salas et al., 2008). The situation is even less clear regarding the neuronal receptors of TeNT. Indeed, TeNT binds the glycoprotein Thy-1 but this interaction is unlikely to be crucial for the biological activity of TeNT as Thy-1 knockout mice retain sensitivity to this toxin (Herreros et al., 2001). Furthermore, a recent report suggested the possibility that both the oligosaccharide and sialic binding sites of TeNT recognize different ganglioside species (Chen et al., 2008).

Following systemic intoxication, the initial target of TeNT and BoNTs is the NMJ. TeNT is internalized via a clathrin-dependent mechanism (Deinhardt et al., 2006a) and transported back to the motor neuron (MN) soma via retrograde axonal transport. It is then further transcytosed to inhibitory interneurons making synaptic contact with MNs, where it exerts its toxic effects (see below). In contrast, BoNTs mainly remain at the NMJ. They are internalized in synaptic vesicles and after acidification of the vesicle lumen are translocated into the neuronal cytosol, where they cleave their target SNAREs. However, several central nervous system (CNS) effects have been reported following peripheral administration of BoNT/A, raising the issue of how these central actions might arise. The next two paragraphs examine in detail the fate of TeNT and BoNTs upon application at the NMJ and their remote effects on brain circuits.

1. Retrograde transport of TeNT in motor neurons

Following peripheral administration, TeNT has been reported to enter the CNS very efficiently. The development of fluorescently-tagged versions of the TeNT H_C binding fragment has provided one important tool for characterizing the mechanisms of the retrograde transport of TeNT in MNs (Lalli et al., 2003a). Indeed, TeNT and TeNT H_C are internalized and transported within MNs in morphologically identical organelles with overlapping speed distributions (Lalli et al., 2003b).

TeNT enters synaptic terminals of MNs via clathrin-coated pits and axolemmal infoldings associated with lipid microdomains (Roux et al., 2005). Specifically, TeNT H_C binds to a lipid-protein receptor complex containing the ganglioside GD1b. TeNT is then laterally sorted to clathrin-coated pits and, during this sorting event, GD1b is excluded from the toxin receptor complex (Deinhardt et al., 2006a). TeNT endocytosis requires the activity of dynamin and a subset of classical clathrin endocytic adaptors, including AP-2, and AP180, but it is independent of epsin-1 (Deinhardt et al., 2006a). After clathrin-mediated endocytosis, TeNT is sorted towards the retrograde transport route. Recent data indicate that two small GTPases, Rab5 and Rab7, are required in a sequential manner for the sorting steps preceding retrograde translocation of TeNT-positive cargoes (Deinhardt et al., 2006b). TeNT appears to first transit through a Rab5-positive, stationary compartment, and then progresses to a Rab7-positive moving compartment. Functional knockdown of either Rab5 or Rab7 completely abolishes TeNT retrograde transport in MNs (Deinhardt et al., 2006b).

The progression of TeNT-positive organelles along axons requires the concerted activity of both cytoplasmic dynein (a microtubule-based motor) and myosin Va (an F-actin based motor) (Lalli et al., 2003b). Interestingly, the transport pathway of TeNT is shared by the neurotrophin receptors $p75^{NTR}$ and TrkB, as well as their ligands nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) (Deinhardt et al., 2006b). TeNT-positive axonal carriers also contain phosphorylated Erk1/2, indicating that they might have signalling capability (Deinhardt et al., 2006b; G.S., unpublished results). This is consistent with the observation that TeNT and TeNT H_C mediate activation of intracellular signalling pathways involving neurotrophin receptors (Gil et al., 2003), although the details of this transactivation mechanism remain unclear.

It is interesting to note that retrogradely moving TeNT-positive carriers exhibit a neutral pH, which is kept constant during transport (Bohnert and Schiavo, 2005). This is in contrast to the situation found in the classical endosomal pathway, which undergoes rapid acidification upon internalization. This specialized pH regulation of TeNT carriers is likely to be due to the exclusion of the