such as, *e.g.*, inhibition of neurotransmitter release in neurons not targeted for toxin treatment or paralysis of a muscle not targeted for treatment. For example, a patient administered a therapeutically effective amount of a BoNT/A treatment into the neck muscles for torticollis may develop dysphagia because of dispersal of the toxin into the oropharynx. Thus, there remains a need for improved Clostridial toxins and/or Clostridial toxin chimeras that are effective at the site of treatment, but have negligible to minimal effects in areas not targeted for toxin treatment.

[05] The growing clinical, therapeutic, and cosmetic use of Clostridial toxins and Clostridial toxin chimeras in therapies requiring larger doses necessitates the pharmaceutical industry to develop modified Clostridial toxins and Clostridial toxin chimeras that are effective at the target site of application, but reduce or prevent the possible side-effects associated with the dispersal of the toxins to an unwanted location. The present specification provides novel modified Clostridial toxins and Clostridial toxin chimeras that reduce or prevent unwanted side-effects associated with toxin dispersal into non-targeted areas. These and related advantages are useful for various clinical, therapeutic and cosmetic applications, such as, *e.g.*, the treatment of neuromuscular disorders, neuropathic disorders, eye disorders, pain, muscle injuries, headache, cardiovascular diseases, neuropsychiatric disorders, endocrine disorders, cancers, otic disorders and hyperkinetic facial lines, as well as, other disorders where a Clostridial toxin or a Clostridial toxin chimeric administration to a mammal can produce a beneficial effect.

SUMMARY

[05a] In one aspect, there is provided a botulinum neurotoxin variant of SEQ ID NO:1, wherein the botulinum neurotoxin variant comprises a first thrombin cleavage site engineered immediately carboxy-terminal to amino acid N880 and a second thrombin cleavage site engineered immediately carboxy-terminal to amino acid D890, wherein the variant has botulinum neurotoxin activity.

BRIEF DESCRIPTION OF THE DRAWINGS

[06] **FIG. 1** shows a schematic of the current paradigm of neurotransmitter release and Clostridial toxin intoxication in a central and peripheral neuron. FIG. 1A shows a schematic for the neurotransmitter release mechanism of a central and peripheral neuron. The release process can be described as comprising two steps: 1) vesicle docking, where the vesicle-bound SNARE protein of a vesicle containing neurotransmitter molecules associates with the membrane-bound SNARE proteins located at the plasma membrane; and 2) neurotransmitter release, where the vesicle fuses with the plasma membrane and the neurotransmitter molecules are exocytosed. FIG. 1B shows a schematic of the intoxication mechanism for tetanus and botulinum toxin activity in a central and peripheral neuron. This intoxication process can be described as comprising four steps: 1) receptor binding, where a Clostridial toxin binds to a Clostridial receptor system and initiates the intoxication process; 2) complex internalization, where after toxin binding, a vesicle containing the toxin/receptor system complex is endocytosed into the cell; 3) light chain translocation, where multiple events are thought to occur, including, e.g., changes in the internal pH of the vesicle, formation of a channel pore comprising the HN domain of the Clostridial toxin heavy chain, separation of the Clostridial toxin light chain from the heavy chain, and release of the active light chain and 4) enzymatic target modification, where the activate light chain of Clostridial toxin proteolytically cleaves its target SNARE substrate, such as, e.g., SNAP-25, VAMP or Syntaxin, thereby preventing vesicle docking and neurotransmitter release.

[07] **FIG. 2** shows the domain organization of naturally-occurring Clostridial toxins. The single-chain form depicts the amino to carboxyl linear organization comprising an enzymatic domain, a translocation domain, and a binding domain. The di-chain loop region located between the translocation and enzymatic domains is depicted by the double SS bracket. This region comprises an endogenous di-chain loop protease cleavage site that upon proteolytic cleavage with a naturally-occurring protease, such as,