Claims

1. A modified botulinum neurotoxin A (BoNT/A) L-chain protease that demonstrates increased human SNAP-23 (hSNAP-23) cleavage relative to wild-type BoNT/A L-chain (SEQ ID NO: 1), and having a modified amino acid sequence relative to the wild-type BoNT/A L-chain (SEQ ID NO: 1), that comprises:
    a) at least one amino acid residue change located within a first BoNT/A L-chain protease binding pocket for binding to the P182 /D178 binding site of hSNAP-23;
    b) wherein said first BoNT/A L-chain protease binding pocket is defined by amino acid residue E148 of wild-type BoNT/A L-chain (SEQ ID NO: 1);
    c) and wherein said at least one amino acid residue change comprises:
        i. an amino acid residue selected from the group consisting of asparagine and tyrosine at the position on the modified L-chain protease amino acid sequence that corresponds to amino acid residue E148 of wild-type BoNT/A L-chain (SEQ ID NO: 1).

2. The modified BoNT/ A L-chain protease according to claim 1, further comprising:
    a) an amino acid residue change located within a second BoNT/A L-chain protease binding pocket for binding to the D189/D192 binding site of hSNAP-23;
    b) wherein said second BoNT/A L-chain protease binding pocket is defined by amino acid residue Q29 of wild-type BoNT/A L-chain (SEQ ID NO: 1);
    c) and wherein said amino acid residue change comprises:
        i. an amino acid residue selected from the group consisting of alanine at the position on the modified L-chain protease amino acid sequence that corresponds to amino

acid residue Q29 of wild-type BoNT/A L-chain (SEQ ID NO: 1).

3. The modified BoNT/A L-chain protease according to claim 1 or claim 2, further comprising:
    a) an amino acid residue change located within a third BoNT/A L-chain protease binding pocket for binding to the I198 binding site of hSNAP-23;
    b) wherein said third BoNT/A L-chain protease binding pocket is defined by amino acid residue K166 of wild-type BoNT/A L-chain (SEQ ID NO: 1);
    c) and wherein said amino acid residue change comprises:
        i. an amino acid residue selected from the group consisting of phenylalanine and valine at the position on the modified L-chain protease amino acid sequence that corresponds to amino acid residue K166 of wild-type BoNT/A L-chain (SEQ ID NO: 1).

4. The modified BoNT/A L-chain protease according to any one of the preceding claims, further comprising:
    a) at least one amino acid residue change located within a fourth BoNT/A L-chain protease binding pocket for binding to the K185 binding site of hSNAP-23;
    b) wherein said fourth BoNT/A L-chain protease binding pocket is defined by amino acid residue G305 of wild-type BoNT/A L-chain (SEQ ID NO: 1);
    c) and wherein said at least one amino acid residue change comprises:
        i. an amino acid residue selected from the group consisting of glutamate and aspartate at the position on the modified L-chain protease amino acid sequence that corresponds to amino acid residue G305 of wild-type BoNT/A L-chain (SEQ ID NO: 1).

5. The modified BoNT/A L-chain protease according to any one of the preceding claims, further comprising:
    a) an amino acid residue change located within a fifth BoNT/A L-chain protease binding pocket for binding to the R186 binding site of hSNAP-23;
    b) wherein said fifth BoNT/A L-chain protease binding pocket is defined by amino acid residue S143 of wild-type BoNT/A L-chain (SEQ ID NO: 1);
    c) and wherein said amino acid residue change comprises:
        i. an amino acid residue selected from the group consisting of glutamine, glutamate and aspartate at the position on the modified L-chain protease amino acid sequence that corresponds to amino acid residue S143 of wild-type BoNT/A L-chain (SEQ ID NO: 1).

6. The modified BoNT/ A L-chain protease according to any one of the preceding claims, further comprising:
    a) at least one amino acid residue change located within a sixth BoNT/A L-chain protease binding pocket for binding to the K206 binding site of hSNAP-23;
    b) wherein said sixth BoNT/A L-chain protease binding pocket is defined by amino acid residue Y251 of wild-type BoNT/A L-chain (SEQ ID NO: 1);
    c) and wherein said at least one amino acid residue change comprises:
        i. an amino acid residue selected from the group consisting of glutamate and aspartate at the position on the modified L-chain protease amino acid sequence that corresponds to amino acid residue Y251 of wild-type BoNT/A L-chain (SEQ ID NO: 1).

7. The modified BoNT/A L-chain protease according to any one of the preceding claims, further comprising:

a) an amino acid residue change located within a seventh BoNT/A L-chain protease binding pocket for binding to the D210 binding site of hSNAP-23;
b) wherein said seventh BoNT/A L-chain protease binding pocket is defined by amino acid residue S254 of wild-type BoNT/A L-chain (SEQ ID NO: 1);
c) and wherein said amino acid residue change comprises:
    i. an amino acid residue selected from the group consisting of alanine at the position on the modified L-chain protease amino acid sequence that corresponds to amino acid residue S254 of wild-type BoNT/A L-chain (SEQ ID NO: 1).

8. A nucleic acid construct comprising or consisting of a nucleic acid sequence encoding the modified BoNT/A L-chain protease as defined in any preceding Claim.

9. A delivery vehicle, comprising:
    a) the modified BoNT/A L chain protease as defined in any Claims 1 to 7, or the nucleic acid construct of Claim 8; and
    b) means for delivering said modified BoNT/A L chain protease, or said nucleic acid construct, into a target cell, preferably into a non-neuronal target cell.

10. The delivery vehicle according to Claim 7, wherein the means b) for delivering said modified BoNT/A L chain protease to a target cell comprises:
    i) a targeting moiety that binds the delivery vehicle to the target cell; and
    ii) a translocation peptide that translocates the modified BoNT/A L-chain protease or the nucleic acid construct into the target cell, preferably into a non-neuronal target cell.

11. An *in vitro* method of cleaving hSNAP-23, comprising contacting hSNAP-23 *in vitro* or in an *in vitro* cell with a (BoNT/A) L-chain protease according to any of Claims 1 to 7, or with a nucleic acid construct according to Claim 8, or with a delivery vehicle according to Claim 9 or 10.

12. A (BoNT/A) L-chain protease according to any of Claims 1 to 7, or a nucleic acid construct according to Claim 8, or a delivery vehicle according to Claim 9 or 10, for use in a method according to Claim 11.

13. A (BoNT/A) L-chain protease according to any of Claims 1 to 7, or a nucleic acid construct according to Claim 8, or a delivery vehicle according to Claim 9 or 10 for use as a medicament.