molecular weight molecules such as full-length proteins. The active transport of high molecular weight molecules through such barrier often requires specific carriers able to cross the lipid bilayer. Cell penetrating peptides (CPPs) are generally 5–30 amino acids long peptides (or motifs within a peptide) which, for their ability to cross cell membranes, are widely used to deliver proteins, plasmid DNA, RNA, oligonucleotides, liposomes and anti-cancer drugs inside the cells (Borrelli et al., 2018; Bode & Löwik, 2017; Kalafatovic & Giralt, 2017; Kristensen et al., 2016).

SUMMARY

In the present disclosure, it has been discovered that a C-terminal fragment of the CDNF protein surprisingly protects ER stressed sympathetic and dopaminergic neurons *in vitro* and *in vivo,* and in contrast to full-length CDNF the fragment is capable of penetrating neuronal cell membrane as well as the blood-brain-barrier *in vivo*.

Accordingly, it is an aim of the present disclosureto provide a C-terminal CDNF fragment consisting of at least 50 consecutive amino acid residues of the sequence as set forth in SEQ ID NO:1:

MPAMKICEKL KKLDSQICEL KYEKTLDLAS VDLRKMRVAE LKQILHSWGE ECRACAEKTD YVNLIQELAP KYAATHPKTE L

or a sequence which preferably has at least 90 % homology or sequence identity with the sequence of SEQ ID NO:1.

In one aspect, the present disclosure refers to a C-terminal CDNF fragment consisting of at least 50 consecutive amino acid residues of the sequence as set forth in SEQ ID NO:1:

MPAMKICEKL KKLDSQICEL KYEKTLDLAS VDLRKMRVAE LKQILHSWGE ECRACAEKTD YVNLIQELAP KYAATHPKTE L

or a sequence which has at least 90 % homology or sequence identity with the sequence of SEQ ID NO:1, wherein said fragment is a cell membrane penetrating peptide and has a protective effect on neuronal cells, for use as a medicine.

In another aspect, the present disclosure refers a pharmaceutical composition comprising the C-terminal CDNF fragment disclosed herein and at least one of the following: physiologically acceptable carrier, buffer, excipient, preservative and stabilizer.

In a further aspect, the present disclosure refers the pharmaceutical composition disclosed herein for use as a medicine.

In another aspect, the present disclosure refers a C-terminal MANF fragment consisting of at least 50 consecutive amino acid residues of the sequence as set forth in SEQ ID NO:2:

ICEKLKKKDS QICELKYDKQ IDLSTVDLKK LRVKELKKIL DDWGETCKGC AEKSDYIRKI NELMPKYAPK AASARTDL

or a sequence which has at least 90 % homology or sequence identity with the sequence of SEQ ID NO:2 for use in the treatment of a degenerative disease or disorder, wherein said fragment is preferably to be administered by intravenous more preferably peripheral administration, intraperitoneal, subcutaneous, intranasal, transdermal, intramuscular, intraocular, or intraarterial administration.

In a further aspect, the present disclosure refers a C-terminal MANF fragment consisting of at least 50 consecutive amino acid residues of the sequence as set forth in SEQ ID NO:2:

ICEKLKKKDS QICELKYDKQ IDLSTVDLKK LRVKELKKIL DDWGETCKGC AEKSDYIRKI NELMPKYAPK AASARTDL

or a sequence which has at least 90 % homology or sequence identity with the sequence of SEQ ID NO:2 for use in the treatment of a type 1 or type 2 diabetes or a retinal disease.

In another aspect, the present disclosure refers a use of C-terminal CDNF fragment comprising or consisting of at least 50 consecutive amino acid residues of the sequence as set forth in SEQ ID NO:1:

MPAMKICEKL KKLDSQICEL KYEKTLDLAS VDLRKMRVAE LKQILHSWGE ECRACAEKTD YVNLIQELAP KYAATHPKTE L

or a sequence which has at least 90 % homology or sequence identity with the sequence of SEQ ID NO:1, in the manufacture of a medicament for treating a degenerative disease or disorder.

In a further aspect, the present disclosure refers a use of C-terminal MANF fragment, comprising or consisting of at least 50 consecutive amino acid residues of the sequence as set forth in SEQ ID NO:2:

ICEKLKKKDS QICELKYDKQ IDLSTVDLKK LRVKELKKIL DDWGETCKGC
AEKSDYIRKI NELMPKYAPK AASARTDL

or a sequence which has at least 90 % homology or sequence identity with the sequence of SEQ ID NO:2, in the manufacture of a medicament for treating a degenerative disease, a type 1 or type 2 diabetes or a retinal disease.

The present disclosurealso provides a pharmaceutical composition comprising a C-terminal CDNF fragment and at least one of the following: physiologically acceptable carrier, buffer, excipient, preservative and stabilizer.

The results of the present disclosurefurther provides said C-terminal CDNF fragment for use in the treatment of a degenerative disease or disorder including a central nervous system (CNS) disease, diabetes or a retinal disease, wherein said CNS disease is preferably selected from the group consisting of Alzheimer’s disease, Parkinson’s disease, Huntington’s disease and other amyloid diseases, multiple system atrophy, amyotrophic lateral sclerosis, frontotemporal lobar degeneration, dementia with Lewy bodies, mild cognitive impairment, traumatic brain injury, peripheral nerve injuries, addiction and stroke.

The present disclosurealso shows that in contrast to the mature MANF protein the C-terminal fragment of MANF (C-MANF) is able to penetrate the cell membrane of dopamine neurons and protects the neurons in culture.

Another aim of the present disclosureis thus to provide a C-terminal MANF fragment, consisting of at least 50 consecutive amino acid residues of the sequence as set forth in SEQ ID NO:2:

ICEKLKKKDS QICELKYDKQ IDLSTVDLKK LRVKELKKIL DDWGETCKGC AEKSDYIRKI NELMPKYAPK AASARTDL

or a sequence which preferably has at least 90 % homology or sequence identity with the sequence of SEQ ID NO:2 for use in the treatment of a degenerative disease or disorder including central nervous system (CNS) diseases, wherein said fragment is administered by intravenous or peripheral administration, intraperitoneal, subcutaneous, intranasal, transdermal, intramuscular, intraocular, or intra-arterial administration.

Also a pharmaceutical composition comprising said C-terminal MANF fragment, and at least one of the following: physiologically acceptable carrier, buffer, excipient and stabilizer, is provided for use in the treatment of a degenerative disease or disorder including central nervous system (CNS) diseases, wherein said fragment is administered by intravenous or peripheral administration, intraperitoneal, subcutaneous, intranasal, transdermal, intramuscular, intraocular, or intra-arterial administration.

Further aim of the present disclosureis to provide a C-terminal MANF fragment consisting of at least 50 consecutive amino acid residues of the sequence as set forth in SEQ ID NO:2:

ICEKLKKKDS QICELKYDKQ IDLSTVDLKK LRVKELKKIL DDWGETCKGC AEKSDYIRKI NELMPKYAPK AASARTDL

or a sequence which preferably has at least 90 % homology or sequence identity with the sequence of SEQ ID NO:2 for use in the treatment of type 1 or type 2 diabetes or a retinal disease.

The present disclosurealso provides a pharmaceutical composition comprising the C-terminal MANF fragment and at least one of the following: physiologically acceptable carrier, buffer, excipient, preservative and stabilizer for use in the treatment of type 1 or type 2 diabetes or a retinal disease.

The aforementioned and other advantages and benefits of the present disclosureare achieved in the manner described as characteristics in the accompanying claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. (A) CDNF has two domains: The N-terminal domain and the C-terminal domain. N-terminal domain can bind oxidized phospholipids (and at least MANF N-terminal domain also lipid sulfatide, also known as 3-O-sulfogalactosylceramide, see Bai et al., 2018) and is a saposin-like domain. The C-terminal domain has the C-X-X-C (i.e. C-R-A-C) sequence and C-terminal ER retention signal KTEL and is a SAPLIP-like domain. CDNF can be proteolytically cleaved *in vitro* yielding these two domains. (B) Schematic view on the structures of MANF and CDNF. Black vertical bars show the location of 8 conserved cysteine residues.

Figure 2. CDNF and C-terminal fragment of CDNF expressed from plasmids rescue ER stressed superior cervical ganglia (SCG) sympathetic neurons. In the experiment, SCG neurons from 7 days old rats/mice were microinjected with indicated plasmids expressing CDNF, the C-terminal fragment of CDNF (C-CDNF), the control plasmid PCR3.1, and positive control when nerve growth factor (NGF at 10 ng/ml) was added to the culture medium. Next day tunicamycin (TM) at 2 µM was added to trigger ER stress-induced cell death, then after three days living and fluorescent neurons were counted and results are disclosed as percentage of initial neurons.

Figure 3. CDNF and CDNF fragment proteins rescue ER stressed SCG neurons when microinjected into the cytoplasm. In the experiment, SCG neurons were prepared from postnatal day 1 old mice, cultured for 7 days, and then injected with the recombinant human CDNF or C-CDNF protein, respectively. Next day tunicamycin (2 µM) was added, and after 3 days the living fluorescent neurons were counted. The results are disclosed as a percentage of initial neurons.

Figure 4. C-terminal fragment of MANF (C-MANF) protects dopaminergic neurons in culture. Dissociated cultures of embryonic day 13 (E13) NMRI mouse midbrain floors were grown on a 96-well plate for 5 days with C-MANF, GDNF added to the culture medium (positive control) or without growth factors as control. The cultures were thereafter stained for tyrosine hydroxylase (TH). Images were scanned by CellInsight™ and immunopositive

CLAIMS

1. A C-terminal CDNF fragment consisting of at least 50 consecutive amino acid residues of the sequence as set forth in SEQ ID NO:1:

MPAMKICEKL KKLDSQICEL KYEKTLDLAS VDLRKMRVAE LKQILHSWGE ECRACAEKTD YVNLIQELAP KYAATHPKTE L

or a sequence which has at least 90 % homology or sequence identity with the sequence of SEQ ID NO:1, wherein said fragment is a cell membrane penetrating peptide and has a protective effect on neuronal cells, for use as a medicine.

2. The fragment for use according to claim 1 in the treatment of a degenerative disease or disorder.

3. The fragment for use according to claim 2, wherein said degenerative disease is a neurodegenerative disease.

4. The fragment for use according to claim 3, wherein said neurodegenerative disease is a central nervous system (CNS) disease selected from the group consisting of: Alzheimer's disease, Parkinson's disease, multiple system atrophy, amyotrophic lateral sclerosis, frontotemporal lobar degeneration, dementia with Lewy bodies, mild cognitive impairment, Huntington's disease, traumatic brain injury, drug addiction and stroke.

5. The fragment for use according to claim 4, wherein said CNS disease is Parkinson's disease.

6. The fragment for use according to claim 2 in the treatment of type I or type II diabetes.

7. The fragment for use according to claim 3 in the treatment of amyotrophic lateral sclerosis.

8. The fragment for use according to claim 2 in the treatment of retinal disorders such as retinitis pigmentosa.

9. The fragment for use according to any of claims 1-8, wherein said fragment is to be administered by intravenous administration, intraperitoneal, subcutaneous, intrathecal, intracerebroventricular, intranasal, transdermal, intracerebral, intramuscular, intraocular, or intraarterial administration or said fragment is to be administered via a viral expression vector.

10. The fragment for use according to claim 9, wherein said intravenous administration is peripheral administration.

11. The fragment for use according to any of the preceding claims, wherein said sequence which has at least 90 % homology or sequence identity with the sequence of SEQ ID NO:1 consists of at least 50 consecutive amino acid residues of the sequence of SEQ ID NO:3:

MPAMKICEKL KKLDSQICEL KYEKTLDLAS VDLRKMRVAE LKQILHSWGE EC**XX**CAEKTD YVNLIQELAP KYAATHPKTE L

wherein X is any amino acid.

12. The fragment for use according to claim 11, wherein said sequence which has at least 90 % homology or sequence identity with the sequence of SEQ ID NO:1 comprises sequence CKGC in positions 52-55 of SEQ ID NO:1.

13. The fragment for use according to any one of claims 1-10, wherein said fragment has the sequence of SEQ ID NO:4:

KYEKTLDLAS VDLRKMRVAE LKQILHSWGE ECRACAEKTD YVNLIQELAP KYAATHPKTE L

or a sequence which has at least 90 % homology or sequence identity with the sequence of SEQ ID NO:4.

14. The fragment for use according to any of claims 11-13, wherein said fragment comprises the C-terminal amino acid L in position 81 of SEQ ID NO:1.

15. The fragment for use according to any of claims 1-13, wherein said fragment lacks the ER retention signal KTEL corresponding to positions 78-81 of SEQ ID NO:1.

16. The fragment for use according to any of the preceding claims, wherein the fragment further comprises modifications protecting the fragment from enzymatic degradation preferably selected from the group consisting of amidation of the C-terminus and acetylation of the N-terminus.

17. The fragment for use according to any of the preceding claims further conjugated to a detectable chemical or biochemical moiety.

18. The fragment for use according to any of the preceding claims, wherein said fragment is a cell-penetrating peptide and is capable of penetrating human blood-brain barrier.

19. A pharmaceutical composition comprising the C-terminal CDNF fragment as defined in any of claims 1-18 and at least one of the following: physiologically acceptable carrier, buffer, excipient, preservative and stabilizer.

20. The pharmaceutical composition according to claim 19 for intravenous administration preferably peripheral administration, intraperitoneal, subcutaneous, intrathecal, intracerebroventricular, intranasal, transdermal, intracerebral, intramuscular, intraocular, or intraarterial administration.

21. The pharmaceutical composition according to claim 19 or 20, wherein the C-terminal CDNF fragment comprises modifications protecting the fragment from enzymatic degradation preferably selected from the group consisting of amidation of the C-terminus and acetylation of the N-terminus.

22. The pharmaceutical composition according to claim 19 or 20, wherein the C-terminal CDNF fragment is conjugated to a detectable chemical or biochemical moiety.

23. The pharmaceutical composition according to any one of claims 19-22 for use as a medicine.

24. The pharmaceutical composition for use according to claim 23 in the treatment of a degenerative disease or disorder.

25. The pharmaceutical composition for use according to claim 24, wherein said degenerative disease or disorder is a central nervous system (CNS) disease selected from the group consisting of: Alzheimer’s disease, Parkinson’s disease, multiple system atrophy, amyotrophic lateral sclerosis, frontotemporal lobar degeneration, dementia with Lewy bodies, mild cognitive impairment, Huntington’s disease, traumatic brain injury, drug addiction and stroke.

26. The pharmaceutical composition according to claim 23 for use in the treatment of type I or type II diabetes.

27. The pharmaceutical composition according to claim 23 for use in the treatment of retinal disorders such as retinitis pigmentosa.

28. A C-terminal MANF fragment consisting of at least 50 consecutive amino acid residues of the sequence as set forth in SEQ ID NO:2:

ICEKLKKKDS QICELKYDKQ IDLSTVDLKK LRVKELKKIL DDWGETCKGC AEKSDYIRKI NELMPKYAPK AASARTDL

or a sequence which has at least 90 % homology or sequence identity with the sequence of SEQ ID NO:2 for use in the treatment of a degenerative disease or disorder, wherein said fragment is preferably to be administered by intravenous more preferably peripheral administration, intraperitoneal, subcutaneous, intranasal, transdermal, intramuscular, intraocular, or intraarterial administration.

29. The fragment for use according to claim 28, wherein said fragment has the sequence of SEQ ID NO:5:

KYDKQ IDLSTVDLKK LRVKELKKIL DDWGETCKGC AEKSDYIRKI NELMPKYAPK AASARTDL

or a sequence which has at least 90 % homology or sequence identity with the sequence of SEQ ID NO:5.

30. The fragment for use according to claim 28 or 29, wherein said fragment comprises the C-terminal amino acid L in position 78 of SEQ ID NO:2.

31. The fragment for use according to claim 28 or 29, wherein said fragment lacks the ER retention signal RTDL corresponding to positions 75-78 of SEQ ID NO:2.

32. The fragment for use according to any of claims 28-31, wherein the fragment further comprises modifications protecting the fragment from enzymatic degradation, preferably selected from the group consisting of amidation of the C-terminus and acetylation of the N-terminus.

33. The fragment for use according to any of claims 28-32 further conjugated to a detectable chemical or biochemical moiety.

34. The fragment for use according to any of claims 28-32, wherein said fragment is a cell-penetrating peptide and is capable of penetrating human blood-brain barrier.

35. The fragment for use according to any of claims 28-34, wherein said degenerative disease is a central nervous system (CNS) disease selected from the group consisting of: Alzheimer's disease, Parkinson's disease, multiple system atrophy, amyotrophic lateral sclerosis, frontotemporal lobar degeneration, dementia with Lewy bodies, mild cognitive impairment, Huntington's disease, traumatic brain injury, drug addiction and stroke.

36. A pharmaceutical composition comprising the C-terminal MANF fragment according to any of claims 28-34, and at least one of the following: physiologically acceptable carrier, buffer, excipient, preservative and stabilizer, for use in the treatment of a central nervous system (CNS) disease wherein said fragment is to be administered by intravenous preferably peripheral administration, intraperitoneal, subcutaneous, intranasal, transdermal, intramuscular, intraocular, or intraarterial administration.

37. A C-terminal MANF fragment consisting of at least 50 consecutive amino acid residues of the sequence as set forth in SEQ ID NO:2:

ICEKLKKKDS QICELKYDKQ IDLSTVDLKK LRVKELKKIL DDWGETCKGC AEKSDYIRKI NELMPKYAPK AASARTDL

or a sequence which has at least 90 % homology or sequence identity with the sequence of SEQ ID NO:2 for use in the treatment of a type 1 or type 2 diabetes or a retinal disease.

38. The fragment for use according to claim 37, wherein said fragment has the sequence of SEQ ID NO:5:

KYDKQ IDLSTVDLKK LRVKELKKIL DDWGETCKGC AEKSDYIRKI NELMPKYAPK AASARTDL

or a sequence which has at least 90 % homology or sequence identity to the sequence of SEQ ID NO:5.

39. The fragment for use according to claim 37 or 38, wherein the fragment further comprises modifications protecting the fragment from enzymatic degradation preferably selected from the group consisting of amidation of the C-terminus and acetylation of the N-terminus.

40. The fragment for use according to any of claims 37-39, wherein said fragment is a cell-penetrating peptide and is capable of penetrating human blood-brain barrier.

41. A pharmaceutical composition comprising the C-terminal MANF fragment according to claim 37 or 38 and at least one of the following: physiologically acceptable carrier, buffer, excipient, preservative and stabilizer for use in the treatment of type 1 or type 2 diabetes or a retinal disease.

42. The pharmaceutical composition for use according to claim 41 for intravenous, preferably peripheral administration.

43. The pharmaceutical composition for use according to claim 41, wherein said composition is to be administered by intravenous, intraperitoneal, subcutaneous, intrathecal, intracerebroventricular, intranasal, transdermal, intracerebral, intramuscular, intraocular, or intraarterial administration.

44. Use of C-terminal CDNF fragment comprising or consisting of at least 50 consecutive amino acid residues of the sequence as set forth in SEQ ID NO:1:

MPAMKICEKL KKLDSQICEL KYEKTLDLAS VDLRKMRVAE LKQILHSWGE ECRACAEKTD YVNLIQELAP KYAATHPKTE L

or a sequence which has at least 90 % homology or sequence identity with the sequence of SEQ ID NO:1, in the manufacture of a medicament for treating a degenerative disease or disorder.

45. Use of C-terminal MANF fragment, comprising or consisting of at least 50 consecutive amino acid residues of the sequence as set forth in SEQ ID NO:2:

ICEKLKKKDS QICELKYDKQ IDLSTVDLKK LRVKELKKIL DDWGETCKGC AEKSDYIRKI NELMPKYAPK AASARTDL

or a sequence which has at least 90 % homology or sequence identity with the sequence of SEQ ID NO:2, in the manufacture of a medicament for treating a degenerative disease, a type 1 or type 2 diabetes or a retinal disease.