

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*.

In one aspect, the present disclosure refers to a C-terminal CDNF fragment comprising or consisting of at least the consecutive amino acid residues at positions 38-70 or 25-57 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 80 % homology or sequence identity with the sequence of positions 38-70 or 25-57 in SEQ ID NO:1, wherein said fragment is a cell membrane

penetrating peptide, preferably capable of penetrating human blood-brain barrier, and has a protective effect on neuronal cells, for use as a medicine.

In another aspect, the present disclosure refers to the fragment for use as described herein in the treatment of a degenerative disease or disorder, preferably a neurodegenerative disease, such as Parkinson's disease or amyotrophic lateral sclerosis. In yet another aspect, the present disclosure refers to a pharmaceutical composition comprising the C-terminal CDNF fragment as defined herein and at least one of the following: physiologically acceptable carrier, buffer, excipient, preservative and stabilizer.

In yet another aspect, the present disclosure refers to a C-terminal CDNF fragment comprising or consisting of at least the consecutive amino acid residues at positions 38-70 or

25-57 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 80 % homology or sequence identity with the sequence of positions 38-70 or 25-57 in SEQ ID NO:1, wherein said fragment is a cell membrane penetrating peptide and has a protective effect on neuronal cells, with a length up to 57, 53, 51, 50, 49, 43, 37 or 33 amino acids, wherein said fragment comprises at least the consecutive amino acid residues at positions 31-70 or 25-57 of the sequence as set forth in SEQ ID NO:1 or a sequence which has at least 80 % homology or sequence identity with the sequence of positions 31-70 or 25-57 in SEQ ID NO:1 and the sequence flanking said consecutive amino acid residues has at least 80 % homology or sequence identity with the sequence at corresponding positions in SEQ ID NO:1.

- 10 In a further aspect, the present disclosure refers to an isolated polynucleotide comprising a nucleotide sequence encoding the C-terminal CDNF fragment consisting of the consecutive amino acid residues at positions 31-73 (peptide 6), 25-73 (peptide 4), 21-73 (peptide 3), 21-70 (peptide 7), 31-81 (peptide 5), or 25-81 (peptide 2), 25-57 (peptide 15), or 37-73 (peptide 8) of the sequence as set forth in SEQ ID NO:1 or a sequence which has at least 80 % homology or sequence identity with the sequence of positions 31-73, 25-73, 21-73, 21-70, 31-81, 25-81, 25-57, or 37-73 in SEQ ID NO:1. In a still further aspect, the present disclosure refers to an expression vector encoding the isolated polynucleotide as described herein. In a still further aspect, the present disclosure refers to a host cell transformed with the vector as described herein.
- 20 Accordingly, it is an aim of the present disclosure to provide a C-terminal CDNF fragment comprising or consisting of at least the consecutive amino acid residues at positions 38-70 or 25-57 of the sequence as set forth in SEQ ID NO:1:

MPAMKICEKL KKLDSQICEL KYEKTLDLAS VDLRKMRVAE LKQILHSWGE
ECRACA EKTD YVNLIQELAP KYAATHPKTE L

- 25 or a sequence which has at least 80 % homology or sequence identity with the sequence of positions 38-70 or 25-57 in SEQ ID NO:1.

The present disclosure also 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.

- 30 The results of the present disclosure further 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.

- 5 The present disclosure also 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 disclosure is thus to provide a C-terminal MANF fragment, preferably with the length of 36-78 amino acids, comprising or consisting of at least the
10 consecutive amino acid residues at positions 33-68 or 19-52 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 80 % homology or sequence identity with the
15 sequence of positions 33-68 or 19-52 in 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.

- 20 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,
25 intramuscular, intraocular, or intra-arterial administration.

Further aim of the present disclosure is to provide a C-terminal MANF fragment, preferably with the length of 36-78 amino acids, comprising or consisting of at least the consecutive amino acid residues at positions 33-68 or 19-52 of the sequence as set forth in SEQ ID NO:2:

ICEKLKKKDS QICELKYDKQ IDLSTVDLKK LRVKELKKIL DDWGETCKGC
30 AEKSDYIRKI NELMPKYAPK AASARTDL

or a sequence which has at least 80 % homology or sequence identity with the sequence of positions 33-68 or 19-52 in SEQ ID NO:2 for use in the treatment of type 1 or type 2 diabetes or a retinal disease.

The present disclosure also 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 disclosure are 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 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 μ M 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 μ M) 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

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CLAIMS

1. A C-terminal CDNF fragment comprising or consisting of at least the consecutive amino acid residues at positions 38-70 or 25-57 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 80 % homology or sequence identity with the sequence of positions 38-70 or 25-57 in SEQ ID NO:1, wherein said fragment is a cell membrane penetrating peptide, preferably capable of penetrating human blood-brain barrier, 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, preferably a neurodegenerative disease, such as Parkinson's disease or amyotrophic lateral sclerosis.

3. The fragment for use according to claim 2, 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.

4. The fragment for use according to claim 2 in the treatment of type I or type II diabetes or retinal disorders such as retinitis pigmentosa.

5. The fragment for use according to any of claims 1-4, wherein said fragment is administered by intravenous administration, intraperitoneal, subcutaneous, intrathecal, intracerebroventricular, intranasal, transdermal, intracerebral, intramuscular, intraocular, or intraarterial administration or said fragment is administered via a viral expression vector and wherein said intravenous administration is preferably peripheral administration.

6. The fragment for use according to any of the previous claims, wherein said fragment comprises at least the consecutive amino acid residues at positions 38-70 or 25-57 of the sequence as set forth in SEQ ID NO:1 or a sequence which has at least 80 % homology or sequence identity with the sequence of positions 38-70 or 25-57 in SEQ ID NO:1, and, if present, the sequence flanking said consecutive amino acid residues preferably has at least 80 % homology or sequence identity with the sequence at corresponding positions in SEQ ID NO:1.

7. The fragment for use according to claim 6, wherein said fragment comprises or consists of at least the consecutive amino acid residues at positions 37-70, 36-70, 35-70, 34-70, 33-70, 32-70 or 31-70 of the sequence as set forth in SEQ ID NO:1 or a sequence which has at least 80 % homology or sequence identity with the sequence of positions 37-70, 36-70, 35-70, 34-70, 33-70, 32-70 or 31-70 in SEQ ID NO:1, and, if present, the sequence flanking said consecutive amino acid residues preferably has at least 80 % homology or sequence identity with the sequence at corresponding positions in SEQ ID NO:1.

8. The fragment for use according to claim 6, wherein said fragment comprises or consists of at least the consecutive amino acid residues at positions 25-57, 25-58, 25-59, 25-60, 25-61, 25-62, 25-63, 25-64, 25-65, 25-66, 25-67, 25-68, 25-69 or 25-70 of the sequence as set forth in SEQ ID NO:1 or a sequence which has at least 80 % homology or sequence identity with the sequence of positions 25-57, 25-58, 25-59, 25-60, 25-61, 25-62, 25-63, 25-64, 25-65, 25-66, 25-67, 25-68, 25-69 or 25-70 in SEQ ID NO:1, and, if present, the sequence flanking said consecutive amino acid residues preferably has at least 80 % homology or sequence identity with the sequence at corresponding positions in SEQ ID NO:1.

9. The fragment for use according to claim 7 or 8, wherein said fragment comprises or consists of at least the consecutive amino acid residues at positions 31-73 (peptide 6; SEQ ID NO: 15), 25-73 (peptide 4; SEQ ID NO: 13), 21-73 (peptide 3; SEQ ID NO: 12), 21-70 (peptide 7; SEQ ID NO: 16), 31-81 (peptide 5; SEQ ID NO: 14), 25-81 (peptide 2; SEQ ID NO: 11), 25-57 (peptide 15, SEQ ID NO:24), or 37-73 (peptide 8, SEQ ID NO:17) of the

sequence as set forth in SEQ ID NO:1 or a sequence which has at least 80 % homology or sequence identity with the sequence of positions 31-73, 25-73, 21-73, 21-70, 31-81, 25-81, 25-57, or 37-73 in SEQ ID NO:1, and, if present, the sequence flanking said consecutive amino acid residues preferably has at least 80 % homology or sequence identity with the sequence at corresponding positions in SEQ ID NO:1.

10. The fragment for use according to claim 9, wherein said fragment comprises or consists of at least the consecutive amino acid residues at positions 31-73 or 25-57 of the sequence as set forth in SEQ ID NO:1 or a sequence which has at least 80 % homology or sequence identity with the sequence of positions 31-73 or 25-57 in SEQ ID NO:1 and, if present, the sequence flanking said consecutive amino acid residues preferably has at least 80 % homology or sequence identity with the sequence at corresponding positions in SEQ ID NO:1.

11. The fragment for use according to any one of the previous claim with a length up to 43-81, preferably 43-61 amino acids.

12. The fragment for use according to any of the preceding claims, wherein the terminals of the fragment comprises further modifications protecting the fragment from enzymatic degradation, preferably the fragment comprises amidation of the C-terminus and acetylation of the N-terminus as further modification.

13. The fragment for use according to any of the preceding claims, wherein said fragment is resistant to protease cleavage, and preferably comprises an elongation, deletion, insertion, substitution or modification such that said elongation, deletion, insertion, substitution or modification abolishes at least one protease cleavage site.

14. A pharmaceutical composition comprising the C-terminal CDFN fragment as defined in any of claims 1 and 6-13 and at least one of the following: physiologically acceptable carrier, buffer, excipient, preservative and stabilizer.

15. The pharmaceutical composition according to claim 14 for intravenous administration preferably peripheral administration, intraperitoneal, subcutaneous, intrathecal, intracerebroventricular, intranasal, transdermal, intracerebral, intramuscular, intraocular, or intraarterial administration or said composition is administered via a viral expression vector.

16. A C-terminal CDFN fragment comprising or consisting of at least the consecutive amino acid residues at positions 38-70 or 25-57 of the sequence as set forth in SEQ ID NO:1:

MPAMKICEKL KKLD SQICEL KYEKTLDLAS VDLRKMRVAE LKQILHSWGE
ECRACAEKTD YVNLIQELAP KYAATHPKTE L

or a sequence which has at least 80 % homology or sequence identity with the sequence of positions 38-70 or 25-57 in SEQ ID NO:1, wherein said fragment is a cell membrane penetrating peptide and has a protective effect on neuronal cells, with a length up to 57, 53, 51, 50, 49, 43, 37 or 33 amino acids, wherein said fragment comprises at least the consecutive amino acid residues at positions 31-70 or 25-57 of the sequence as set forth in SEQ ID NO:1 or a sequence which has at least 80 % homology or sequence identity with the sequence of positions 31-70 or 25-57 in SEQ ID NO:1 and the sequence flanking said consecutive amino acid residues has at least 80 % homology or sequence identity with the sequence at corresponding positions in SEQ ID NO:1.

17. The fragment according to claim 16, wherein said fragment comprises or consists of at least the consecutive amino acid residues at positions 37-70, 36-70, 35-70, 34-70, 33-70, 32-70 or 31-70 of the sequence as set forth in SEQ ID NO:1 or a sequence which has at least 80 % homology or sequence identity with the sequence of positions 37-70, 36-70, 35-70, 34-70, 33-70, 32-70 or 31-70 in SEQ ID NO:1.

18. The fragment according to claim 17, wherein said fragment comprises or consists of at least the consecutive amino acid residues at positions 31-73 (peptide 6), 25-73 (peptide 4), 21-73 (peptide 3), 21-70 (peptide 7), 31-81 (peptide 5), 25-81 (peptide 2), 25-57 (peptide 15), or 37-73 (peptide 8) of the sequence as set forth in SEQ ID NO:1 or a sequence which has at least 80 % homology or sequence identity with the sequence of positions 31-73, 25-73, 21-73, 21-70, 31-81, 25-81, 25-57 or 37-73 in SEQ ID NO:1.

19. An isolated polynucleotide comprising a nucleotide sequence encoding the C-terminal CDNF fragment consisting of the consecutive amino acid residues at positions 31-73 (peptide 6), 25-73 (peptide 4), 21-73 (peptide 3), 21-70 (peptide 7), 31-81 (peptide 5), or 25-81 (peptide 2), 25-57 (peptide 15), or 37-73 (peptide 8) of the sequence as set forth in SEQ ID NO:1 or a sequence which has at least 80 % homology or sequence identity with the sequence of positions 31-73, 25-73, 21-73, 21-70, 31-81, 25-81, 25-57, or 37-73 in SEQ ID NO:1.

20. An expression vector encoding the isolated polynucleotide according to claim 19.

21. A host cell transformed with the vector according to claim 20.