

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 a use of a C-terminal CDNF fragment with a

length of up to 49 amino acids 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

or a sequence which has at least 80 % 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 capable of penetrating human blood-brain barrier, and has a protective effect on neuronal cells in the manufacture of a medicament for treating a degenerative disease or disorder, type I or type II diabetes or retinal disorders.

In 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

with a length up to 49 amino acids 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

or a sequence which has at least 80 % 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, 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 % 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 % sequence identity with the sequence at corresponding positions in SEQ ID NO:1.

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), 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 % sequence identity with the sequence of positions 31-73, 25-73, 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.

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
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.

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.

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

CLAIMS

1. Use of a C-terminal CDNF fragment with a length up to 49 amino acids 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 KKLDSEQICEL KYEKTLDLAS VDLRKMRVAE
LKQILHSWGE ECRACAEKTD YVNLIQELAP KYAATHPKTE L

or a sequence which has at least 80 % 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 capable of penetrating human blood-brain barrier, and has a protective effect on neuronal cells, in the manufacture of a medicament for treating a degenerative disease or disorder, type I or type II diabetes or retinal disorders.

2. The use according to claim 1, wherein said degenerative disease or disorder is a neurodegenerative disease, such as Parkinson's disease or amyotrophic lateral sclerosis.

3. The 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 use according to claim 2, wherein said retinal disorder is retinitis pigmentosa.

5. The use according to any of claims 1-4, 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 and wherein said intravenous administration is preferably peripheral administration.

6. The 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 % 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 % sequence identity with the sequence at corresponding positions in SEQ ID NO:1.

7. The 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 % 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 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 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), 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, 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 % sequence identity with the sequence at corresponding positions in SEQ ID NO:1.

10. The 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 % 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 % sequence identity with the sequence at corresponding positions in SEQ ID NO:1.

11. The use according to any one of the previous claims, wherein the length of the fragment is up to 43 amino acids.

12. The 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 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 CDNF 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 to be administered via a viral expression vector.

16. A C-terminal CDNF fragment with a length up to 49 amino acids 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 % 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, 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 % 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 % 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 % 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), 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 % sequence identity with the sequence of positions 31-73, 25-73, 25-57 or 37-73 in SEQ ID NO:1.

19. An isolated polynucleotide comprising a nucleotide sequence encoding the C-terminal CDFN fragment consisting of the consecutive amino acid residues at positions 31-73 (peptide 6), 25-73 (peptide 4), 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 % sequence identity with the sequence of positions 31-73, 25-73, 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.