SUMMARY

Provided herein are ANP polypeptides that bind to and agonize NPR-A and have natriuretic, diuretic and vasorelaxant activity. Moreover, the ANP polypeptides described herein have extended duration of action at NPR-A allowing for dosing as infrequently as once-a-day, thrice-weekly, twice-weekly or once-a-week. The ANP polypeptides described herein also exhibit desirable developability profiles making them suitable for use in therapeutic applications. In this manner, the ANP polypeptides described herein can be useful in chronic treatment to lower blood pressure, reduce pathological wall stress and improve adverse cardiac remodeling, as well as have beneficial effects on lung congestion.

Thus, the present disclosure also provides methods of using ANP polypeptides to treat or prevent cardiovascular disease (CVD) and related conditions, including in particular Heart Failure (HF). Preferred ANP polypeptides and methods of the present disclosure reduce the risk of CV-related death or HF-related hospitalization, reduce the risk of myocardial infarction (MI) or stroke, reduce the probability of a need for left ventricular assist device (LVAD) or cardiac transplant, improve cardiac function and structure, and/or improve the symptoms and physical limitations associated with HF, leading to improvements in QoL.

In an aspect, provided herein is a polypeptide comprising:

$X_1X_2X_3RSSCFX_9X_{10}X_{11}IX_{13}RIGX_{17}X_{18}SGLGCPSX_{26}RX_{28}X_{29}$ (SEQ ID NO:3),

wherein:

X_1 is absent, S or E,

X_2 is absent, L, K, 4-Pal, H or E,

X_3 is absent, R, β-Ala, P, K, E or G,

X_9 is G, 4-Pal or H,

X_{10} is G, K, R, or Dap,

X_{11} is R, K, G or Dap,

X_{13} is D or G,

X_{17} is A, H, Dap, K, R or Orn,

X_{18} is Q, Y or 4-Pal,

X_{26} is F or L,

X_{28} is Y, H or 4-Pal and

X_{29} is either absent or selected from

GGP,

SGAPPPE (SEQ ID NO:4),

KITAKEDE (SEQ ID NO:5),

GPSSGAPPPE (SEQ ID NO:6),

GPSSGAPPPS (SEQ ID NO:7),

GGSSGAPPPS (SEQ ID NO:8),

GGPSSGAPPPS (SEQ ID NO:9),

KGPSSGAPPPS (SEQ ID NO:10),

GGKSSGAPPPS (SEQ ID NO:11),

GGPPS-Aib-KPPPK (SEQ ID NO:12),

GSPSSGAPPPS (SEQ ID NO:13),

RITAREDKQGYA (SEQ ID NO:14),

RITAREDKQGEA (SEQ ID NO:15),

GSPSSGAPPPS-PEG24-G (SEQ ID NO:16),

SGSPSSGAPPPSG (SEQ ID NO:17),

GGESSGEPPPSEE (SEQ ID NO:18),

GSGSPSSGAPPPSG (SEQ ID NO:19), and

SGSPSSGAPPPSEEEG (SEQ ID NO:20)

and the C-terminal amino acid is optionally amidated.

or a pharmaceutically acceptable salt thereof.

In one example, provided herein is a polypeptide of Formula I comprising:

$X_1X_2X_3RSSCFX_9X_{10}X_{11}IX_{13}RIGX_{17}X_{18}SGLGCPSX_{26}RX_{28}X_{29}$ (SEQ ID NO:3),

wherein:

X_1 is absent, S or E,

X_2 is absent, L, K, 4-Pal, H or E,

X_3 is absent, R, β-Ala, P, K, E or G,

X_9 is G, 4-Pal or H,

X_{10} is G, K, R or Dap,

X_{11} is R, K, G or Dap,

X_{13} is D or G,

X_{17} is A, H, Dap, K, R or Orn,

X_{18} is Q, Y or 4-Pal,

X_{26} is F or L,

X_{28} is Y, H or 4-Pal, and

X_{29} is either absent or selected from

GGP,

SGAPPPE (SEQ ID NO:4),

KITAKEDE (SEQ ID NO:5),

GPSSGAPPPE (SEQ ID NO:6),

GPSSGAPPPS (SEQ ID NO:7),

GGSSGAPPPS (SEQ ID NO:8),

GGPSSGAPPPS (SEQ ID NO:9),

KGPSSGAPPPS (SEQ ID NO:10),

GGKSSGAPPPS (SEQ ID NO:11),

GGPPS-Aib-KPPPK (SEQ ID NO:12),

GSPSSGAPPPS (SEQ ID NO:13),

RITAREDKQGYA (SEQ ID NO:14),

RITAREDKQGEA (SEQ ID NO:15),

GSPSSGAPPPS-PEG24-G (SEQ ID NO:16),

SGSPSSGAPPPSG (SEQ ID NO:17),

GGESSGEPPPSEE (SEQ ID NO:18),

GSGSPSSGAPPPSG (SEQ ID NO:19), and

SGSPSSGAPPPSEEEG (SEQ ID NO:20)

and the C-terminal amino acid is optionally amidated,

or a pharmaceutically acceptable salt thereof.

In some examples, the polypeptide contains a disulfide linkage between the cysteines present at positions 7 and 23 (C7 and C23). In some examples, the polypeptide contains a thioacetal linkage between the cysteines present at positions 7 and 23 (C7 and C23).

In another example, a polypeptide of Formula I, or a pharmaceutically acceptable salt thereof, is conjugated to a fatty acid. For instance, in some examples, the polypeptide of Formula I, or a pharmaceutically acceptable salt thereof, further comprises a fatty acid conjugated to the amino acid present at the N terminus of the

polypeptide and comprises a basic structure from an amino-terminus (N-terminus) to a carboxy-terminus (C-terminus) of Formula II:

fatty acid-Z1-Z2-Z3-$X_1X_2X_3$RSSCF$X_9X_{10}X_{11}$IDRIG$X_{17}X_{18}$SGLGCX_{24}SX_{26}R$X_{28}X_{29}$ (SEQ ID NO:21),

wherein the fatty acid is a C_{16}-C_{26} fatty acid and is conjugated to the amino acid present at the N terminus of the polypeptide through a structure Z1-Z2-Z3, wherein

Z1 comprises an amino acid selected from γGlu, E and β-Ala,

Z2 is either absent or comprises a four to ten amino acid sequence comprising amino acids independently selected from E, K, G, P, A and S, and

Z3 is either absent or comprises a polyethylene glycol (PEG) or a (2-[2-(2-amino-ethoxy)-ethoxy]-acetyl) moiety.

In some examples, Z1 is an amino acid selected from γGlu, E and β-Ala.

In some examples, Z2 is selected from APPSG, $(EK)_bG$, $(EP)_bG$, $K(EK)_cG$, and $(EK)_cE$, wherein b is 2, 3 or 4 and c is 1, 2, 3 or 4. For example, in some examples, Z2 is EKEKEKG (SEQ ID NO:22), EPEPEPG (SEQ ID NO:23), APPSG (SEQ ID NO:24), KEKEKG (SEQ ID NO:25) or EKEKEKE (SEQ ID NO:26).

In some examples, Z3 is selected from $(\text{polyethylene glycol})_m$ wherein m is a whole number selected from 10 to 30 and $((2\text{-}[2\text{-}(2\text{-amino-ethoxy})\text{-ethoxy}]\text{-acetyl}))_n$ wherein n is selected from 2 to 10. For example, in some examples, Z3 is $(\text{polyethylene glycol})_{12}$ or $(\text{polyethylene glycol})_{24}$ or $((2\text{-}[2\text{-}(2\text{-amino-ethoxy})\text{-ethoxy}]\text{-acetyl}))_4$ or $(2\text{-}[2\text{-}(2\text{-amino-ethoxy})\text{-ethoxy}]\text{-acetyl}))_6$ or $(2\text{-}[2\text{-}(2\text{-amino-ethoxy})\text{-ethoxy}]\text{-acetyl}))_8$.

In another example, a pharmaceutical composition is provided that includes a polypeptide, or a pharmaceutically acceptable salt thereof, as described herein and a pharmaceutically acceptable carrier, diluent or excipient.

In another aspect, provided herein is a pharmaceutical composition comprising the polypeptide as described herein or a pharmaceutically acceptable salt thereof, and at least one pharmaceutically acceptable carrier, diluent, or excipient.

In another example, provided herein is a method for using a polypeptide or a pharmaceutically acceptable salt thereof described herein to treat or prevent a cardiovascular disease (CVD) and related conditions. Such methods can include at least a step of administering to an individual in need thereof an effective amount of a polypeptide described herein, or a pharmaceutically acceptable salt thereof. In some instances, the CVD

is heart failure (HF), in particular it is Heart Failure with preserved Ejection Factor (HfpEF).

In another example, a polypeptide, or a pharmaceutically acceptable salt thereof, as described herein is provided for use in therapy.

In another aspect, provided herein is the polypeptide as described herein, or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition as described herein, for use in therapy. In another example, a polypeptide, or a pharmaceutically acceptable salt thereof, as described herein is provided for use in treating or preventing a CVD. In some instances, the CVD is HF, in particular it is HfpEF.

In another aspect, provided herein is the polypeptide as described herein, or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition as described herein, for use in treating a cardiovascular disease (CVD).

In another aspect, provided herein is the polypeptide as described herein, or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition as described herein, for use in treating heart failure.

In another aspect, provided herein is the polypeptide as described herein, or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition as described herein, for use in treating HFpEF.

In another example, a polypeptide, or a pharmaceutically acceptable salt thereof, as described herein is provided for use in manufacturing a medicament for treating or preventing a CVD. In some instances, the CVD is HF, in particular it is HfpEF.

In another aspect, provided herein is use of the polypeptide as described herein, or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition as described herein, in the manufacture of a medicament for treating a CVD.

In another aspect, provided herein is use of the polypeptide as described herein, or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition as described herein, in the manufacture of a medicament for treating heart failure.

In another aspect, provided herein is use of the polypeptide as described herein, or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition as described herein, in the manufacture of a medicament for treating HFpEF.

DETAILED DESCRIPTION

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of skill in the art to which the disclosure pertains. Although any methods and materials similar to or equivalent to those described herein can be used in the practice or testing of the ANP polypeptides, pharmaceutical compositions, and methods, the preferred methods and materials are described herein.

Moreover, reference to an element by the indefinite article “a” or “an” does not exclude the possibility that more than one element is present, unless the context clearly requires that there be one and only one element. The indefinite article “a” or “an” thus usually means “at least one.”

As used herein, “about” means within a statistically meaningful range of a value or values such as, for example, a stated concentration, length, molecular weight, pH, sequence identity, time frame, temperature or volume. Such a value or range can be within an order of magnitude typically within 20%, more typically within 10%, and even more typically within 5% of a given value or range. The allowable variation encompassed by “about” will depend upon the particular system under study, and can be readily appreciated by one of skill in the art.

As used herein, and in reference to one or more of the ANP receptors, “activity,” “activate,” “activating” and the like means a capacity of a compound, such as ANP

CLAIMS

The invention claimed is:

1. A polypeptide comprising:

$X_1X_2X_3RSSCFX_9X_{10}X_{11}IX_{13}RIGX_{17}X_{18}SGLGCPSX_{26}RX_{28}X_{29}$ (SEQ ID NO:3), wherein:

X_1 is absent, S or E,

X_2 is absent, L, K, 4-Pal, H or E,

X_3 is absent, R, β-Ala, P, K, E or G,

X_9 is G, 4-Pal or H,

X_{10} is G, K, R, or Dap,

X_{11} is R, K, G or Dap,

X_{13} is D or G,

X_{17} is A, H, Dap, K, R or Orn,

X_{18} is Q, Y or 4-Pal,

X_{26} is F or L,

X_{28} is Y, H or 4-Pal and

X_{29} is either absent or selected from

GGP,

SGAPPPE (SEQ ID NO:4),

KITAKEDE (SEQ ID NO:5),

GPSSGAPPPE (SEQ ID NO:6),

GPSSGAPPPS (SEQ ID NO:7),

GGSSGAPPPS (SEQ ID NO:8),

GGPSSGAPPPS (SEQ ID NO:9),

KGPSSGAPPPS (SEQ ID NO:10),

GGKSSGAPPPS (SEQ ID NO:11),

GGPPS-Aib-KPPPK (SEQ ID NO:12),

GSPSSGAPPPS (SEQ ID NO:13),

RITAREDKQGYA (SEQ ID NO:14),

RITAREDKQGEA (SEQ ID NO:15),

GSPSSGAPPPS-PEG24-G (SEQ ID NO:16),
SGSPSSGAPPPSG (SEQ ID NO:17),
GGESSGEPPPSEE (SEQ ID NO:18),
GSGSPSSGAPPPSG (SEQ ID NO:19), and
SGSPSSGAPPPSEEEG (SEQ ID NO:20)

and the C-terminal amino acid is optionally amidated.

or a pharmaceutically acceptable salt thereof.

2. The polypeptide of claim 1, or a pharmaceutically acceptable salt thereof, comprising a disulfide linkage or a thioacetal linkage between cysteine at position 7 and cysteine at position 23 of SEQ ID NO:3.

3. The polypeptide of claim 1 or 2, or a pharmaceutically acceptable salt thereof, further comprising a fatty acid conjugated to the amino acid present at the N terminus of the polypeptide, and comprising a structure:

fatty acid-Z1-Z2-Z3-$X_1X_2X_3$RSSCF$X_9X_{10}X_{11}$IDRIG$X_{17}X_{18}$SGLGCPSX_{26}R$X_{28}X_{29}$,

wherein the fatty acid is a C_{16}-C_{26} fatty acid and is conjugated to the amino acid present at the N terminus of the polypeptide through a structure Z1-Z2-Z3, wherein

Z1 is an amino acid selected from γGlu, E and β-Ala,

Z2 is selected from $(EK)_bG$, $(EP)_bG$, $K(EK)_cG$, $(EK)_cE$ and APPSG (SEQ ID NO:24), wherein b is 2, 3 or 4 and c is 1, 2, 3 or 4, and

Z3 is either absent or is selected from $(\text{polyethylene glycol})_m$ wherein m is a whole number selected from 10 to 30 or $((2\text{-}[2\text{-}(2\text{-amino-ethoxy})\text{-ethoxy}]\text{-acetyl}))_n$ wherein n is selected from 1 to 10.

4. The polypeptide of any one of claims 1 to 3, or a pharmaceutically acceptable salt thereof, wherein
X_1 is S or E,
X_2 is K or 4-Pal,
X_3 is R, β-Ala or K,
X_9 is G,
X_{10} is G or K,
X_{11} is R or K,
X_{13} is D or G,
X_{17} is H,
X_{18} is Q or Y,
X_{26} is F,
X_{28} is H, and
X_{29} is selected from GGPSSGAPPPS (SEQ ID NO:9), GGKSSGAPPPS (SEQ ID NO:11), and GSPSSGAPPPS (SEQ ID NO:13).

5. The polypeptide of claim 3, or a pharmaceutically acceptable salt thereof, wherein the fatty acid is a C_{16}-C_{22} fatty acid.

6. The polypeptide of claim 5, or a pharmaceutically acceptable salt thereof, wherein the Z1 is γ-Glu.

7. The polypeptide of claim 6, or a pharmaceutically acceptable salt thereof, wherein the Z2 comprises a sequence selected from EKEKEKG (SEQ ID NO:22), EPEPEPG (SEQ ID NO:23) and APPSG (SEQ ID NO:24).

8. The polypeptide of claim 7, or a pharmaceutically acceptable salt thereof, wherein the Z3 is absent or selected from $(\text{polyethylene glycol})_m$ wherein m is 12 or 24, and $((2\text{-}[2\text{-}(2\text{-amino-ethoxy})\text{-ethoxy}]\text{-acetyl}))_n$ wherein n is 4, 6 or 8.

9. The polypeptide of any one of claims 1 to 8 or a pharmaceutically acceptable salt thereof, wherein the polypeptide is selected from SEQ ID NO: 38 to 167.

10. The polypeptide of any one of claims 1 to 9, or a pharmaceutically acceptable salt thereof, wherein the polypeptide is selected from SEQ ID NO: 45, 50, 51, 78, 83, 84, 97, 98, 144, 158 and 159.

11. The polypeptide of any one of claims 1 to 10 or a pharmaceutically acceptable salt thereof, wherein the C terminal is amidated.

12. The polypeptide of any one of claims 1 to 11, or a pharmaceutically acceptable salt thereof, wherein the polypeptide is an agonist of NPR-A.

13. A pharmaceutical composition comprising the polypeptide of any one of claims 1 to 12 or a pharmaceutically acceptable salt thereof, and at least one pharmaceutically acceptable carrier, diluent, or excipient.

14. The pharmaceutical composition of claim 13, wherein the composition is formulated for subcutaneous (SQ) or intravenous (IV) administration.

15. The pharmaceutical composition of claim 14, wherein the composition is formulated for SQ administration.

16. The polypeptide of any one of claims 1 to 12, or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition of claim 13, for use in therapy.

17. The polypeptide of any one of claims 1 to 12, or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition of claim 13, for use in treating a cardiovascular disease (CVD).

18. The polypeptide of any one of claims 1 to 12, or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition of claim 13, for use in treating heart failure.

19. The polypeptide of any one of claims 1 to 12, or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition of claim 13, for use in treating HFpEF.

20. Use of the polypeptide of any one of claims 1 to 12, or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition of claim 13, in the manufacture of a medicament for treating a CVD.

21. Use of the polypeptide of any one of claims 1 to 12, or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition of claim 13, in the manufacture of a medicament for treating heart failure.

22. Use of the polypeptide of any one of claims 1 to 12, or a pharmaceutically acceptable salt thereof, or the pharmaceutical composition of claim 13, in the manufacture of a medicament for treating HFpEF.