US 20110021429A1

(19) **United States**

(12) **Patent Application Publication**
Gaudreau et al.

(10) **Pub. No.: US 2011/0021429 A1**

(43) **Pub. Date: Jan. 27, 2011**

(54) RENOPROTECTION BY GROWTH HORMONE-RELEASING HORMONE AND AGONISTS

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(21) Appl. No.: **12/285,282**

(22) Filed: **Oct. 1, 2008**

Related U.S. Application Data

(60) Provisional application No. 60/960,477, filed on Oct. 1, 2007, provisional application No. 61/006,057, filed on Dec. 17, 2007.

Publication Classification

(51) **Int. Cl.**

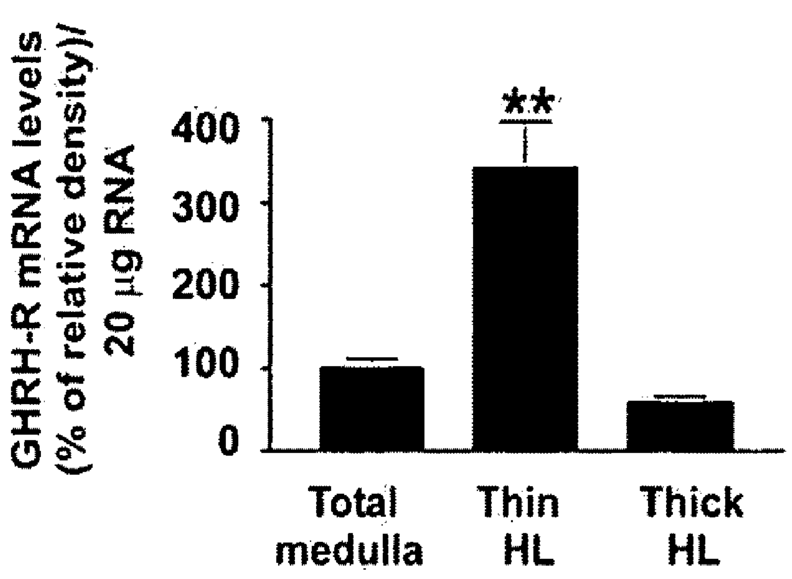
A61K 38/25	(2006.01)
A61K 38/16	(2006.01)
A61P 13/12	(2006.01)
C07K 14/00	(2006.01)
C07K 16/00	(2006.01)
C07H 21/04	(2006.01)
C12N 15/63	(2006.01)
C12N 5/00	(2006.01)
G01N 33/53	(2006.01)

(52) **U.S. Cl.** **514/11.2**; 514/15.4; 530/350; 530/387.9; 536/23.1; 435/320.1; 435/325; 436/501

(57) ABSTRACT

The present invention relates to renoprotection by growth hormone-releasing hormone and agonists. More specifically, the present invention relates to methods for protecting a mammal against oxidative renal damage, of promoting regeneration of kidney cells in a mammal in need thereof and/or of preventing the death of kidney cells due to oxidative stress. The present invention also relates to the identification of rat and human renal GHRH-R sequences.

Figure 1


GHRH-R mRNA levels
(% of relative density)/
20 µg RNA
400
300
200
100
0
**
Total medulla
Thin HL
Thick HL

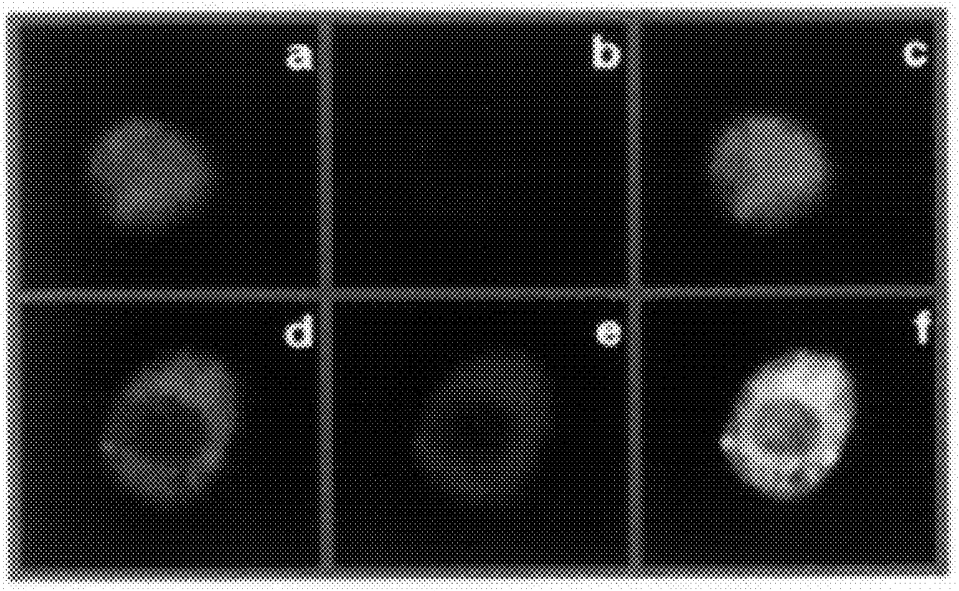


Figure 2

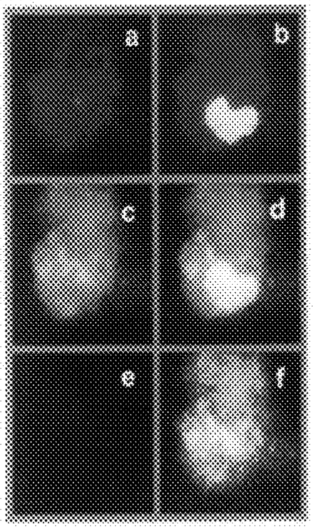


Figure 3A

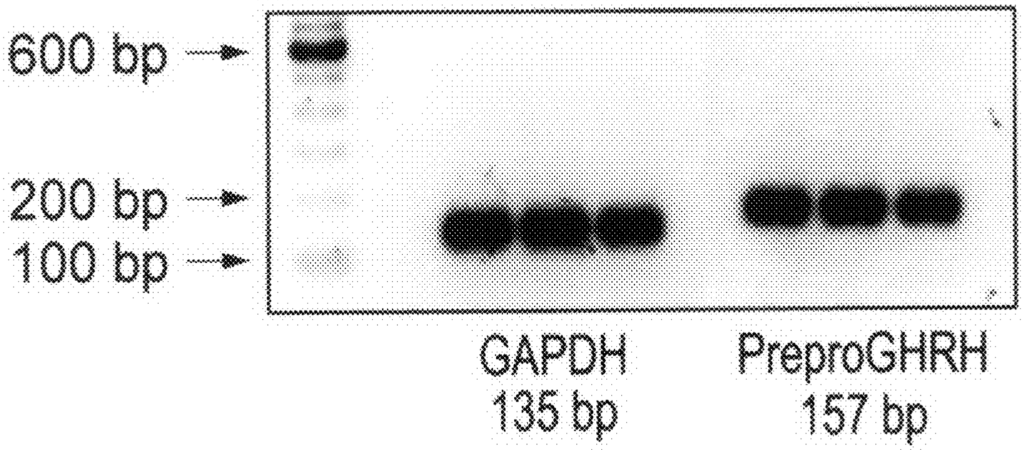


Figure 3B

Figure 4

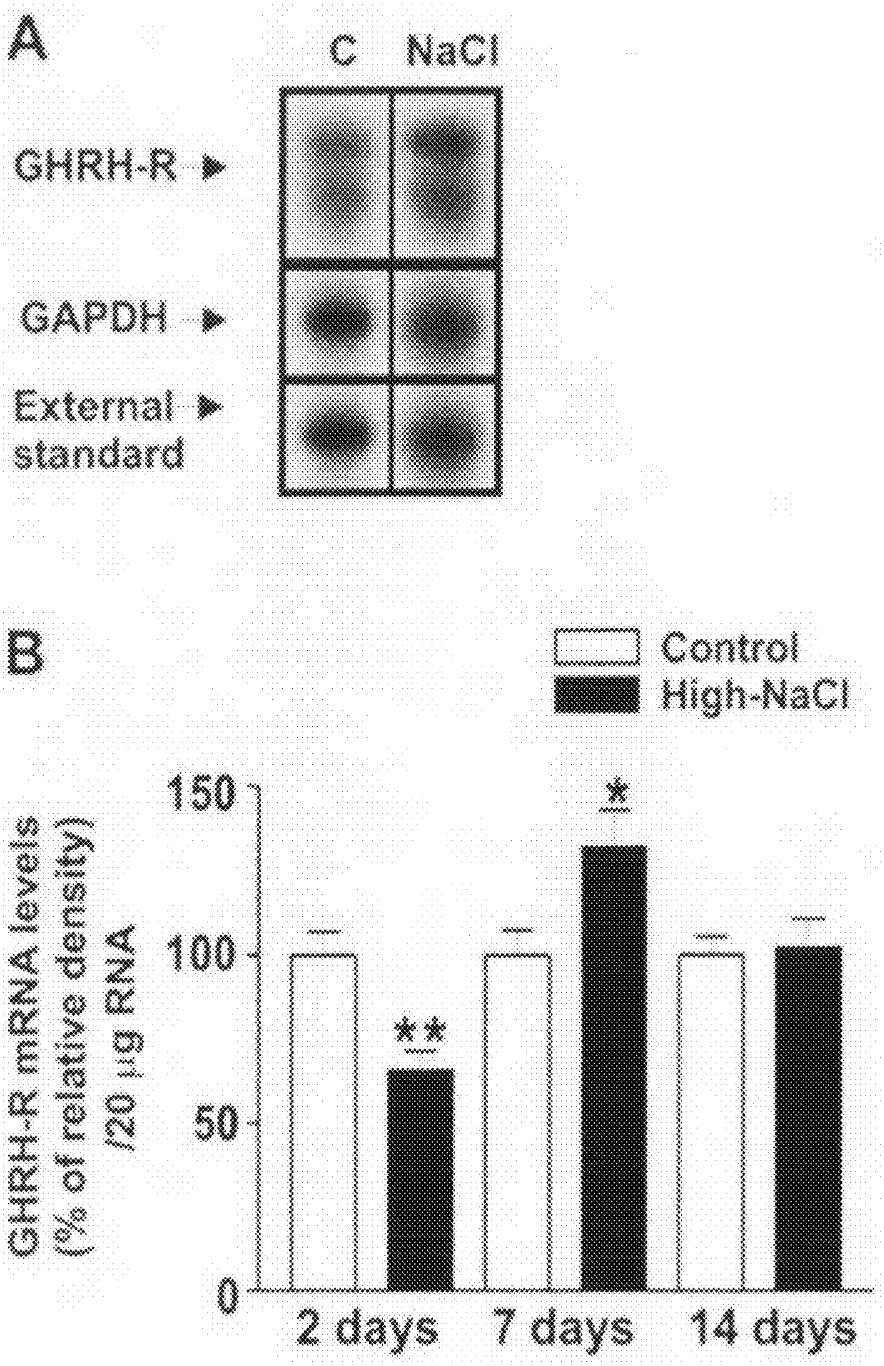

A
C
NaCl
GHRH-R
GAPDH
External standard
B
Control
High-NaCl
GHRH-R mRNA levels (% of relative density) /20 μg RNA
150
100
50
0
**
*
2 days
7 days
14 days

Figure 5

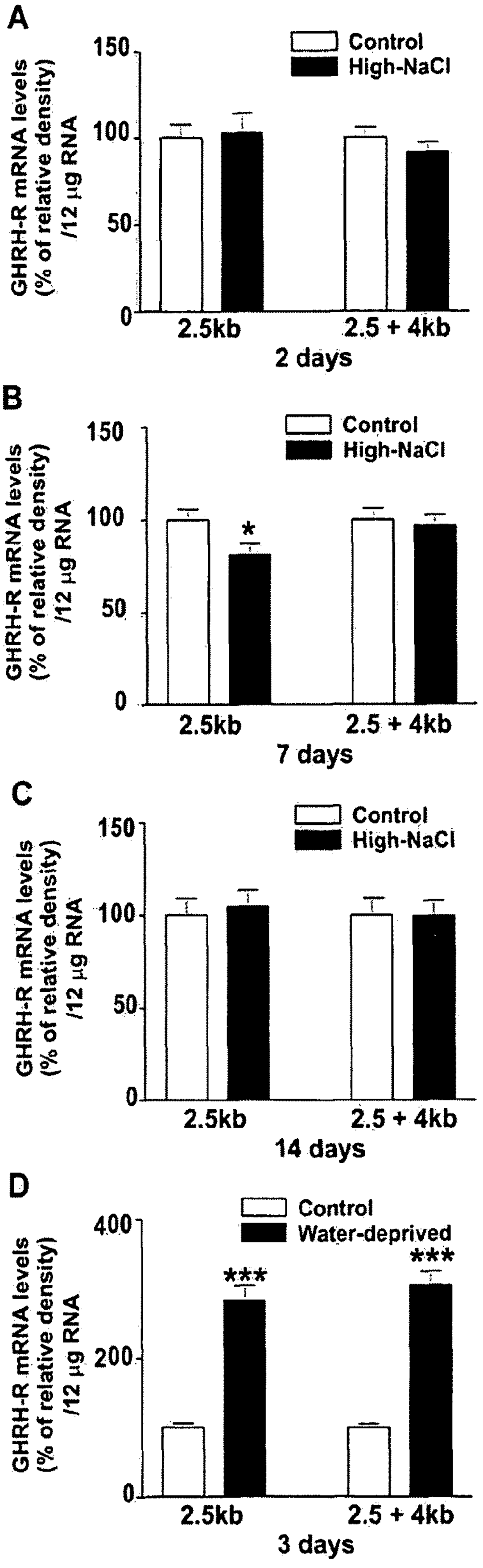

A
150
100
50
0
Control
High-NaCl
GHRH-R mRNA levels (% of relative density) /12 μg RNA
2.5kb
2.5 + 4kb
2 days
B
150
100
50
0
Control
High-NaCl
*
GHRH-R mRNA levels (% of relative density) /12 μg RNA
2.5kb
2.5 + 4kb
7 days
C
150
100
50
0
Control
High-NaCl
GHRH-R mRNA levels (% of relative density) /12 μg RNA
2.5kb
2.5 + 4kb
14 days
D
400
200
0
Control
Water-deprived
***
***
GHRH-R mRNA levels (% of relative density) /12 μg RNA
2.5kb
2.5 + 4kb
3 days

Figure 6

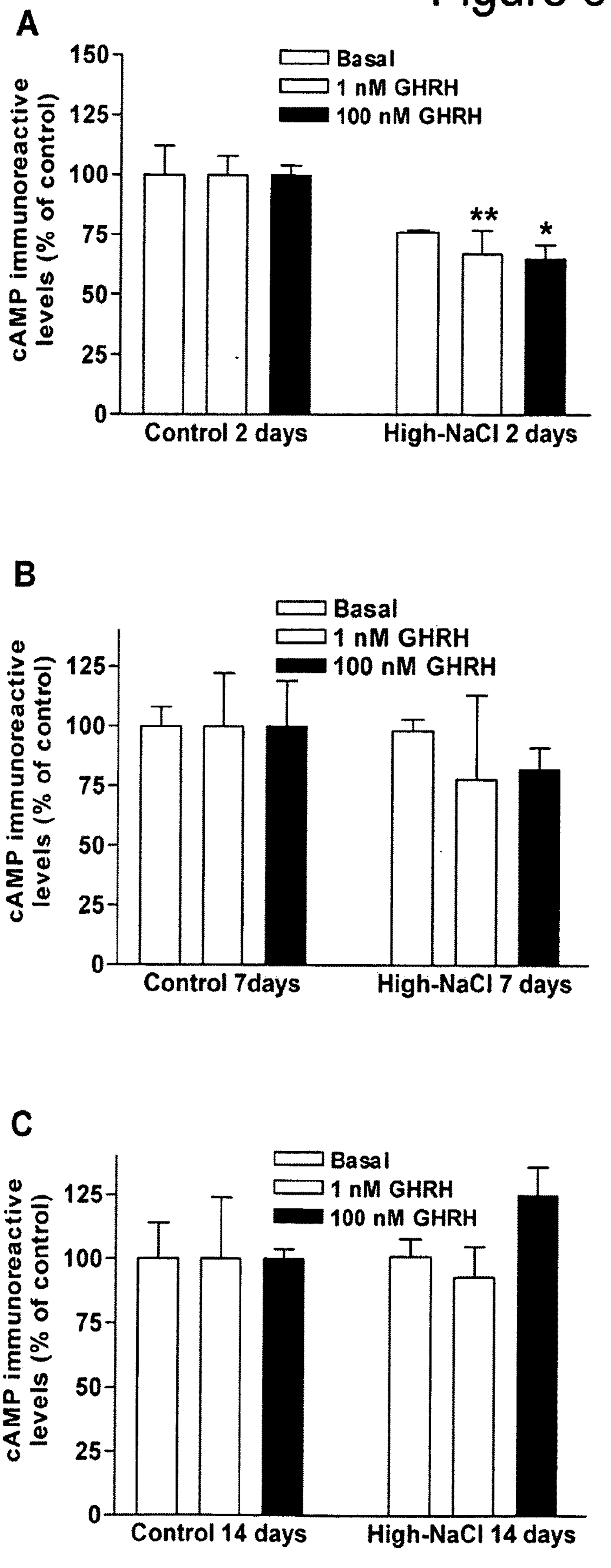
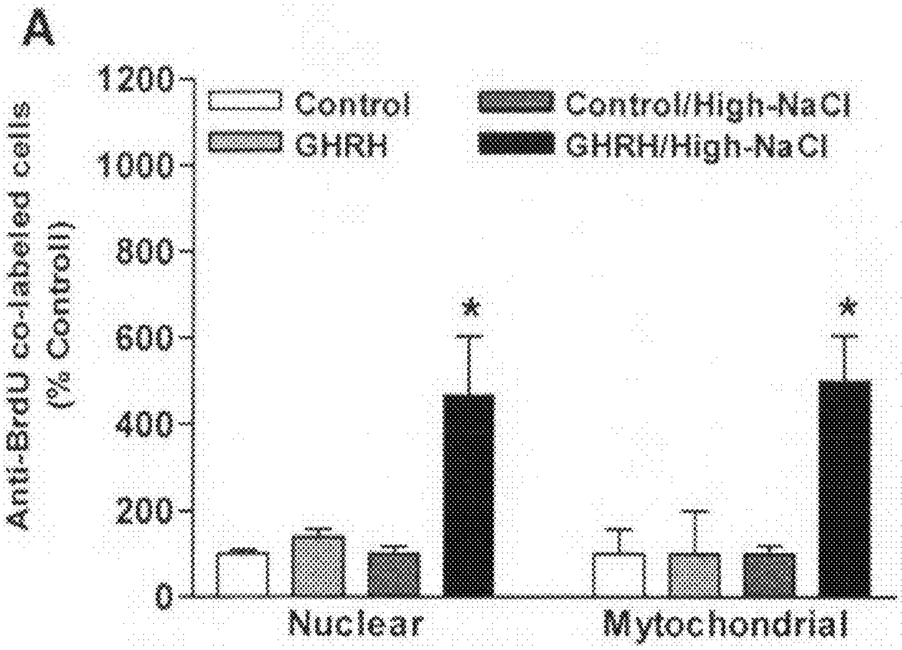

A
cAMP immunoreactive levels (% of control)
150
125
100
75
50
25
0
Basal
1 nM GHRH
100 nM GHRH
**
*
Control 2 days
High-NaCl 2 days
B
cAMP immunoreactive levels (% of control)
125
100
75
50
25
0
Basal
1 nM GHRH
100 nM GHRH
Control 7days
High-NaCl 7 days
C
cAMP immunoreactive levels (% of control)
125
100
75
50
25
0
Basal
1 nM GHRH
100 nM GHRH
Control 14 days
High-NaCl 14 days

Figure 7


A
Anti-BrdU co-labeled cells
(% Controll)
1200
1000
800
600
400
200
0
Control
GHRH
Control/High-NaCl
GHRH/High-NaCl
*
*
Nuclear
Mytochondrial

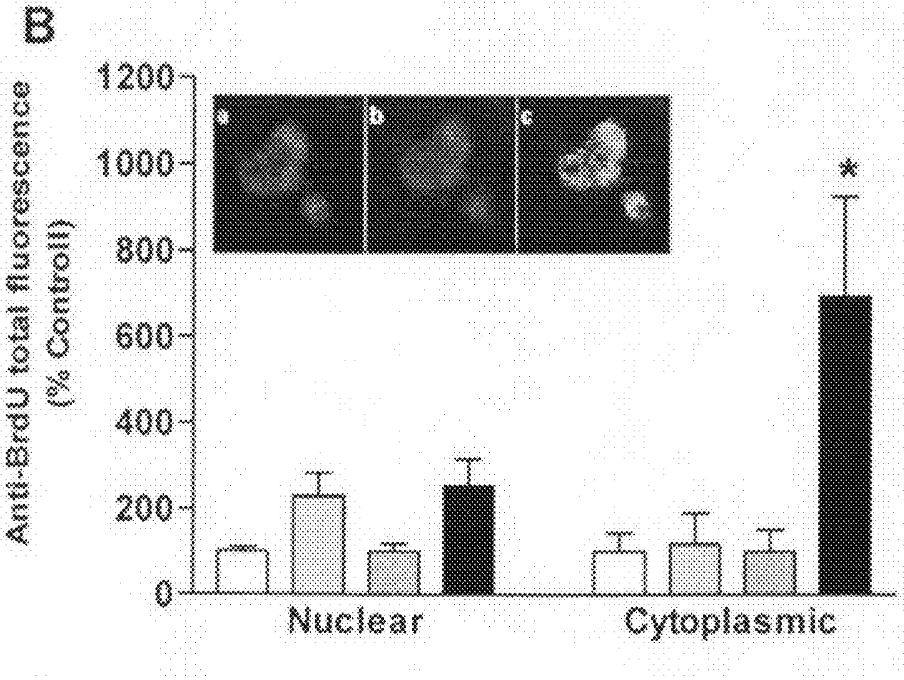

B
Anti-BrdU total fluorescence
(% Controll)
1200
1000
800
600
400
200
0
a
b
c
*
Nuclear
Cytoplasmic

Figure 8

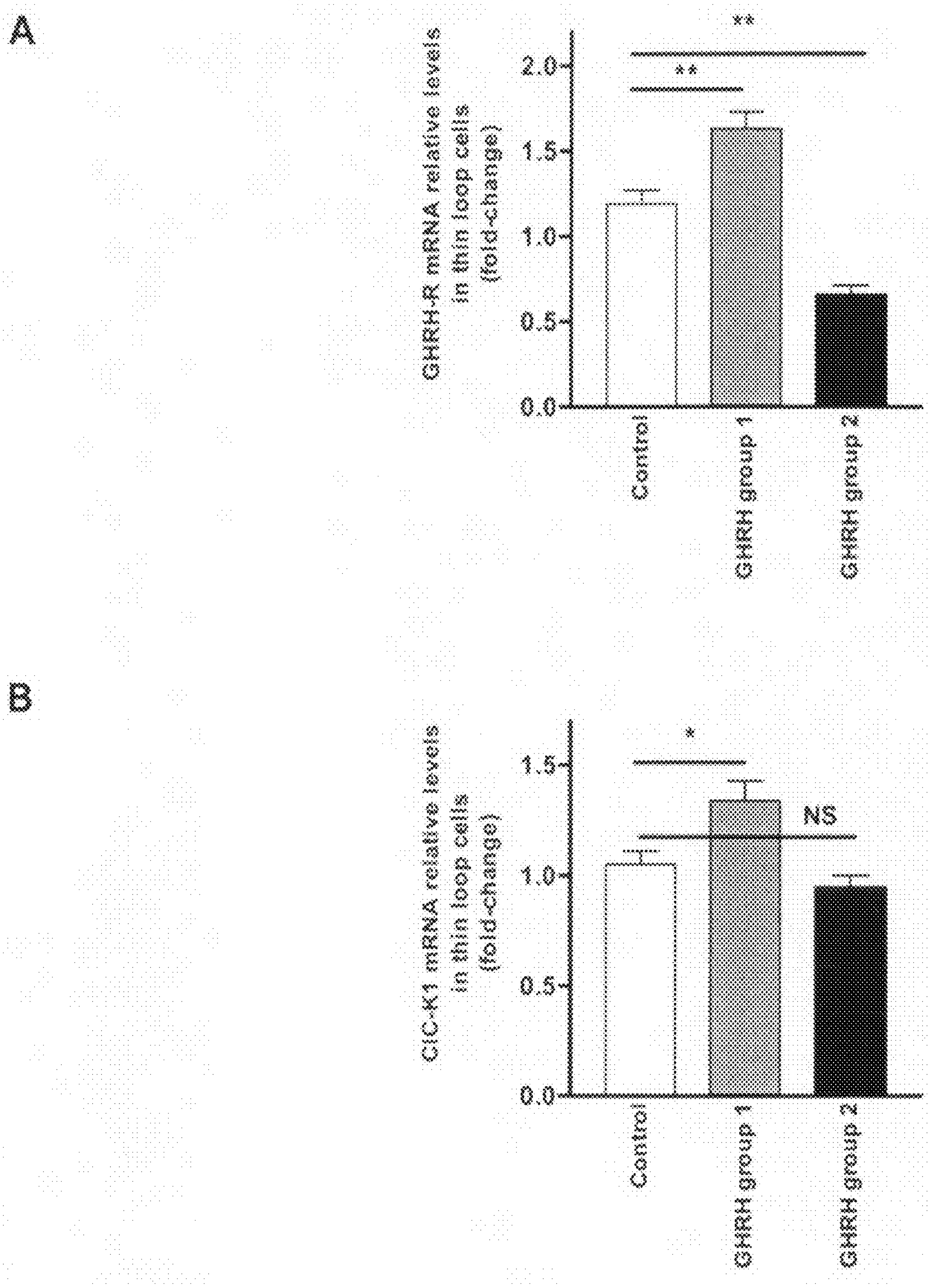

A
GHRH-R mRNA relative levels in thin loop cells (fold-change)
0.0
0.5
1.0
1.5
2.0
**
**
Control
GHRH group 1
GHRH group 2
B
ClC-K1 mRNA relative levels in thin loop cells (fold-change)
0.0
0.5
1.0
1.5
*
NS
Control
GHRH group 1
GHRH group 2

Figure 8

C

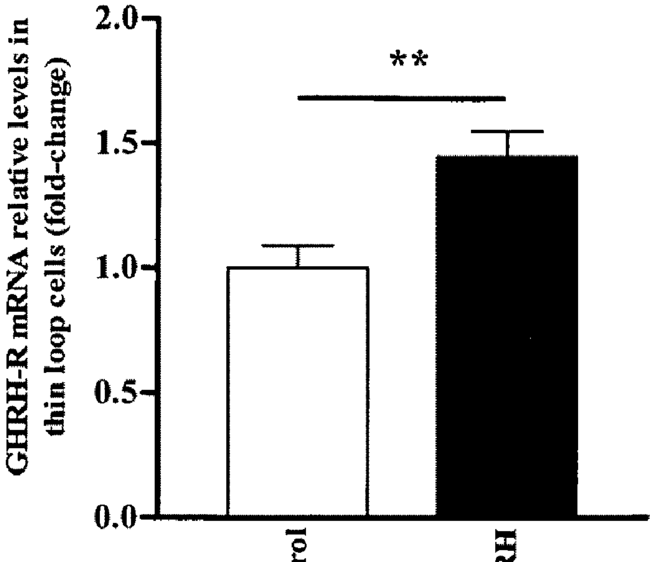
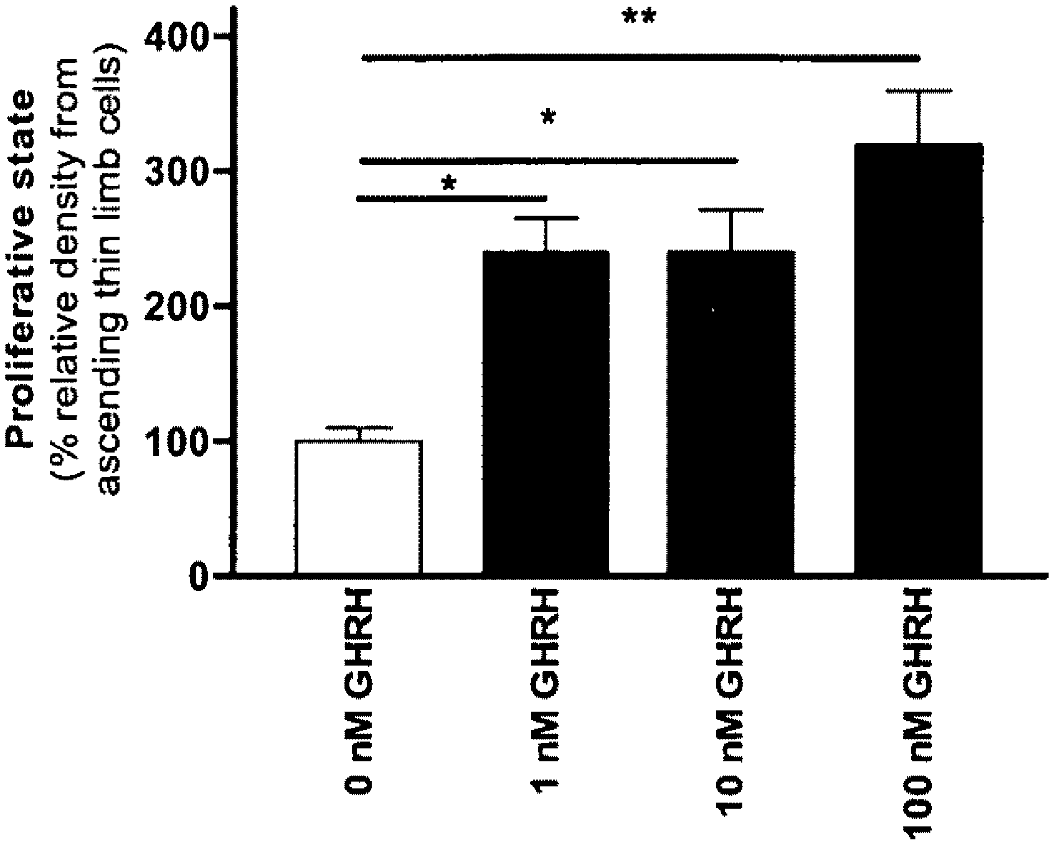

GHRH-R mRNA relative levels in thin loop cells (fold-change)
2.0
1.5
1.0
0.5
0.0
**
Control
rGHRH

Figure 9


Proliferative state
(% relative density from ascending thin limb cells)
400
300
200
100
0
**
*
*
0 nM GHRH
1 nM GHRH
10 nM GHRH
100 nM GHRH

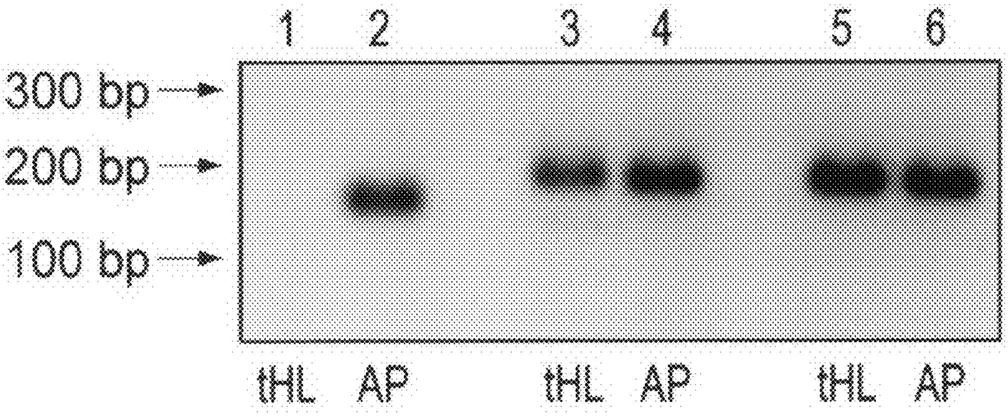


Figure 10A

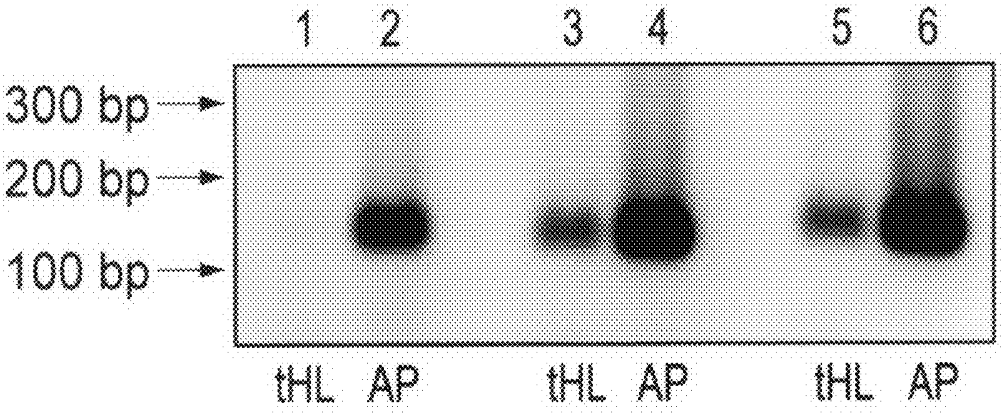


Figure 10B

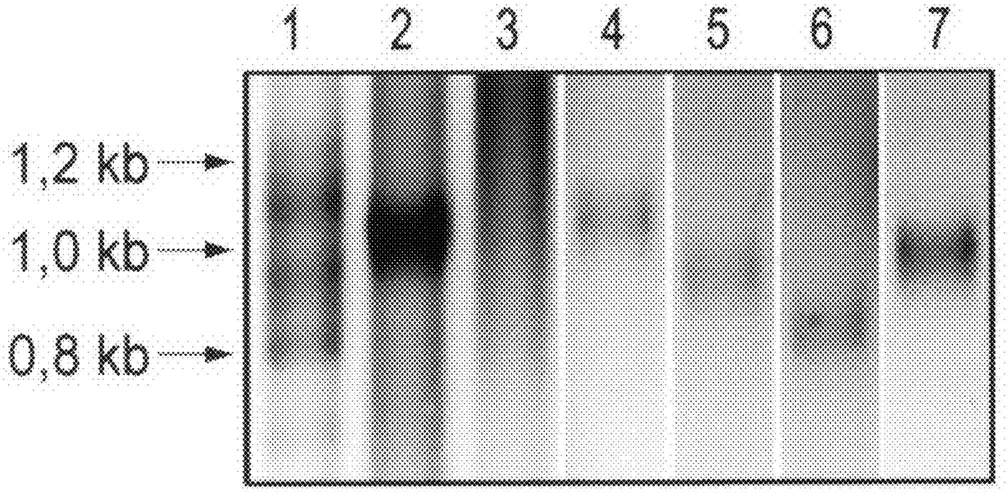


Figure 11A

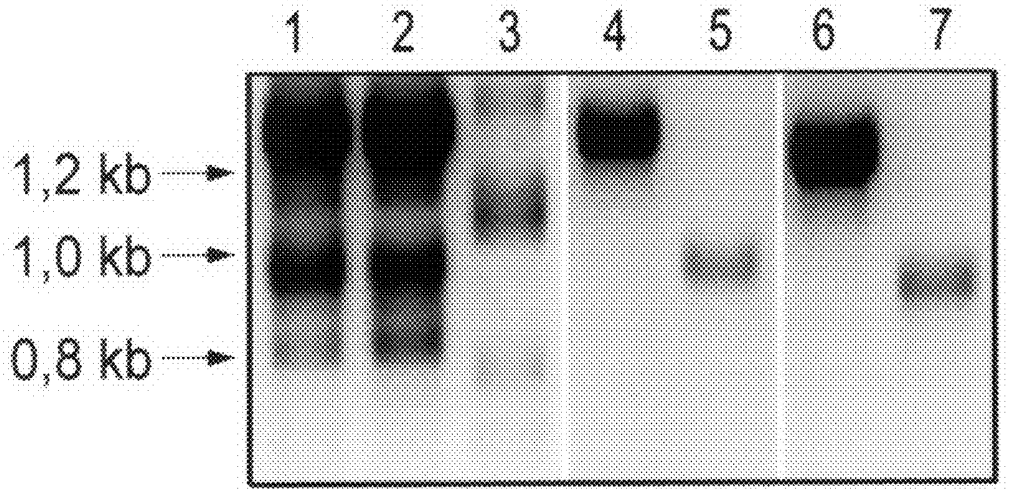


Figure 11B

Figure 12

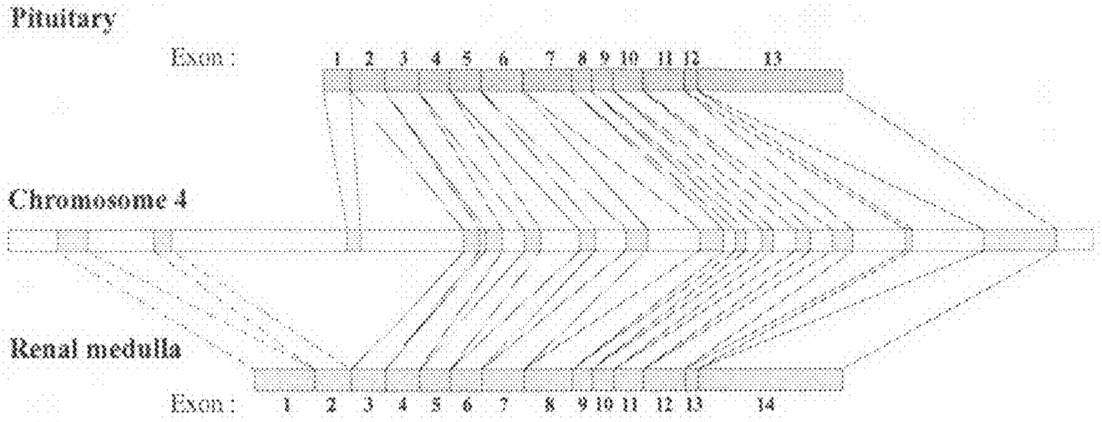

Pituitary
Exon :
1
2
3
4
5
6
7
8
9
10
11
12
13
Chromosome 4
Renal medulla
Exon :
1
2
3
4
5
6
7
8
9
10
11
12
13
14

Figure 13

```
ATTGCACAGCTGCCAGTGGCCTCCTGACCAGCGAGGGGCAGCGCATCTGCCTCCAGGAATGTAGGGCTCAG
AGACACGGGACCAGAAATCCTCAGGGAGGCTGTGGCTTTGCTTCCAGAAGATCCTGAGAGCTCCAGTGTCC
ACTTCTTGCCATCCTTCTGTATGAGTGGGAAACCAACCCAGGCCACCCTTGGGGGCAGGTCCTGGGACTTC
TTGGAGCAGATGAGCCTTTATCCATGCGACAGAACAGGAGGTACCTACTGACACCCATGTCCTAGGATAGA
CACATCTGTATGTGGGTGCgagGCACTGGGTCACCTCCACCTAGAATGTGACTTCATCACTCAGCTGAGAG
ACGATGAGCCTTGCATGCCTTCAGGCGGCAGAGGGGACCAACAACTCGTCCATGGGATGCCCTGGGACCTGG
GATGGGCTGCTGTGCTGGCCCCCAACTGGCTCTGGCCAGTGGGTCTCCCTCCCCTGCCCTGAATTCTTCTC
TCATTTTGGCTCAGACCCAGGGGCTGTGAAAAGGGACTGCACCATCACGGGTTGGTCTGATCCCTTCCCAC
CATATCCCGTGGCCTGTCCTGTGCCCTTGGAACTGCTAACAGAGGAGAAGTCTTACTTCTCCACGGTGAAG
ATCATCTACACCACAGGCCACAGCATCTCCATTGTAGCCCTCTGCGTGGCTATTGCCATCCTGGTTGCTCT
CAGGAGGCTCCACTGCCCCAGGAACTACATCCACACGCAGCTGTTTGCTACTTTCATCCTCAAGGCCAGTG
CTGTGTTCCTGAAGGATGCTGCTGTCTTCCAGGGTGATAGCACGGACCACTGCAGCATGTCCACTATTCTG
TGCAAGGTCTCTGTGGCCGTCTCACATTTTGCCACCATGACCAACTTCAGCTGGCTGCTGGCAGAAGCCGT
CTACCTGAGCTGTCTGTTGGCCTCCACATCTCCTAGGTCCAAACCAGCTTTCTGGTGGCTGGTTCTCGCTG
GCTGGGGACTCCCTGTGCTATGCACTGGTACGTGGGTGGGCTGCAAACTGGCTTTTGAGGACACTGCGTGC
TCGGACCTAGACGACAGCTCCCCCTACTGGTGGATCATCAAAGGGCCCATAGTCCTCTCTGTTGGCGTGAA
CTTTGGGCTAATTTCTCAATATAATTTGCATCCTGCTGAGGAAGCTGGGGCCTGCACAAGGCGGCTTACACA
CACGGGCTCAGTACTGGCGGCTTTCCAAATCAACACTTCTCCTTATCCCGCTGTTTGGAATTCATTACATC
ATCTTCAACTTCCTGCCTGACAGTGCTGGCCTTGGCATCCGTCTACCCCTGGAGCTGGGACTGGGGTCCTT
CCAGGGTTTTGTTGTTGCTGTCCTCTACTGCTTCCTCAATCAAGAGGTGAGGACGGAGATTTCACGCAAAT
GGTATGGCCATGACCCTGAACTTCTGCCAGCTCGGCGGACCTGCACTGAGTGGACCACACCTCCCCGATCC
AGAGTGAAGGTGCTCACCTCTGAGTGCTAGGCCAGCCATCACAAAGGCCGAGCCCCAAAACCCTGCACTCA
AACTGCCATGCCACCAAGGGCAACAAGGTCCTCCCTTCCGTTCTCGTTCTCTGCATCTGCTTTCTCTAGGT
CCCTGTATACCAACCTCCGACTTTCTCAGTTCCTGTATGCCCCCATCTGTTCTTTCTTCCTATCTAGGGCT
ATTGCCCAAGGCCCAGGGAAACCAATAAACTTGTACATGAGTGATCTGCAGTTGAGTCAATGTGGCTCTGA
AGGGGACCTCTTGTCAGCAGCCATTATTTGCACTTCCGGTGCATTCCTCATCCCTTGGCTGCAGCTGCCTC
ATTCCATTTGTCTCTATATGAATTCTCATGTTTTATTTATTTTTTAAAAGCACTCTTTTGTCTCAACCCC
CACTCCCCTTCCCCTCCTCCCGCAGGCAAAATGTGAATCTTTGCTTCTCTGTCTTGCAAGTGTGGAGTGTA
CCACATGTCTGTATTTGGATGT[illegible]TGCTATCTGTTATGTG
```

nucleotides varying in comparison to the genomic sequence			Impact on the protein sequence
Nucleotide in the rat renal sequence	Frequency of occurence	Nucleotide in the rat genomic sequence	
G	3/3	T	None
A	7/9	G	None : identity of resulting codon
G	10/12	A	None

Figure 14

```
 406 atgggatgccctgggacctgggatgggctgctgtgctggcccccca
     M  G  C  P  G  T  W  D  G  L  L  C  W  P  P
 451 actggctctggccagtgggtctccctcccctgccctgaattcttc
     T  G  S  G  Q  W  V  S  L  P  C  P  E  F  F
 496 tctcattttggctcagacccaggggctgtgaaaagggactgcacc
     S  H  F  G  S  D  P  G  A  V  K  R  D  C  T
 541 atcacgggttggtctgatcccttcccaccatatcccgtggcctgt
     I  T  G  W  S  D  P  F  P  P  Y  P  V  A  C
 586 cctgtgcccttggaactgctaacagaggagaagtcttacttctcc
     P  V  P  L  E  L  L  T  E  E  K  S  Y  F  S
 631 acggtgaagatcatctacaccacaggccacagcatctccattgta
     T  V  K  I  I  Y  T  T  G  H  S  I  S  I  V
 676 gccctctgcgtggctattgccatcctggttgctctcaggaggctc
     A  L  C  V  A  I  A  I  L  V  A  L  R  R  L
 721 cactgccccaggaactacatccacacgcagctgtttgctactttc
     H  C  P  R  N  Y  I  H  T  Q  L  F  A  T  F
 766 atcctcaaggccagtgctgtgttcctgaaggatgctgctgtcttc
     I  L  K  A  S  A  V  F  L  K  D  A  A  V  F
 811 cagggtgatagcacggaccactgcagcatgtccactattctgtgc
     Q  G  D  S  T  D  H  C  S  M  S  T  I  L  C
 856 aaggtctctgtggccgtctcacattttgccaccatgaccaacttc
     K  V  S  V  A  V  S  H  F  A  T  M  T  N  F
 901 agctggctgctggcagaagccgtctacctgagctgtctgttggcc
     S  W  L  L  A  E  A  V  Y  L  S  C  L  L  A
 946 tccacatctcctaggtccaaaccagctttctggtggctggttctc
     S  T  S  P  R  S  K  P  A  F  W  W  L  V  L
 991 gctggctggggactccctgtgctatgcactggtacgtgggtgggc
     A  G  W  G  L  P  V  L  C  T  G  T  W  V  G
1036 tgcaaactggcttttgaggacactgcgtgctgggacctagacgac
     C  K  L  A  F  E  D  T  A  C  W  D  L  D  D
1081 agctccccctactggtggatcatcaaagggcccatagtcctctct
     S  S  P  Y  W  W  I  I  K  G  P  I  V  L  S
1126 gttggggtgaactttgggctatttctcaatataatttgcatcctg
     V  G  V  N  F  G  L  F  L  N  I  I  C  I  L
1171 ctgaggaagctggggcctgcacaaggcggcttacacacacgggct
     L  R  K  L  G  P  A  Q  G  G  L  H  T  R  A
1216 cagtactggcggctttccaaatcaacacttctccttatcccgctg
     Q  Y  W  R  L  S  K  S  T  L  L  L  I  P  L
1261 tttggaattcattacatcatcttcaacttcctgcctgacagtgct
     F  G  I  H  Y  I  I  F  N  F  L  P  D  S  A
1306 ggccttggcatccgtctacccctggagctgggactggggtccttc
     G  L  G  I  R  L  P  L  E  L  G  L  G  S  F
1351 cagggttttgttgttgctgtcctctactgcttcctcaatcaagag
     Q  G  F  V  V  A  V  L  Y  C  F  L  N  Q  E
1396 gtgaggacggagatttcacgcaaatggtatggccatgaccctgaa
     V  R  T  E  I  S  R  K  W  Y  G  H  D  P  E
1441 cttctgccagctcggcggacctgcactgagtggaccacacctccc
     L  L  P  A  R  R  T  C  T  E  W  T  T  P  P
1486 cgatcgagagtgaaggtgctcacctctgagtgctag 1521
     R  S  R  V  K  V  L  T  S  E  C  *
```

Figure 15

```
Pituitary       MDSLLWATWVLCLLNLWGVALGHLHLECDFITQLRDDELACLQAAEGTNN
Renal           --------------------------------------------------


Pituitary       SSMGCPGTWDGLLCWPPTGSGQWVSLPCPEFFSHFGSDPGAVKRDCTITG
Renal           --MGCPGTWDGLLCWPPTGSGQWVSLPCPEFFSHFGSDPGAVKRDCTITG
                  ************************************************

Pituitary       WSDPFPPYPVACPVPLELLTEEKSYFSTVKIIYTTGHSISIVALCVAIAI
Renal           WSDPFPPYPVACPVPLELLTEEKSYFSTVKIIYTTGHSISIVALCVAIAI
                **************************************************

Pituitary       LVALRRLHCPRNYIHTQLFATFILKASAVFLKDAAVFQGDSTDHCSMSTI
Renal           LVALRRLHCPRNYIHTQLFATFILKASAVFLKDAAVFQGDSTDHCSMSTI
                **************************************************

Pituitary       LCKVSVAVSHFATMTNFSWLLAEAVYLSCLLASTSPRSKPAFWWLVLAGW
Renal           LCKVSVAVSHFATMTNFSWLLAEAVYLSCLLASTSPRSKPAFWWLVLAGW
                **************************************************

Pituitary       GLPVLCTGTWVGCKLAFEDTACWDLDDSSPYWWIIKGPIVLSVGVNFGLF
Renal           GLPVLCTGTWVGCKLAFEDTACWDLDDSSPYWWIIKGPIVLSVGVNFGLF
                **************************************************

Pituitary       LNIICILLRKLGPAQGGLHTRAQYWRLSKSTLLLIPLFGIHYIIFNFLPD
Renal           LNIICILLRKLGPAQGGLHTRAQYWRLSKSTLLLIPLFGIHYIIFNFLPD
                **************************************************

Pituitary       SAGLGIRLPLELGLGSFQGFVVAVLYCFLNQEVRTEISRKWYGHDPELLP
Renal           SAGLGIRLPLELGLGSFQGFVVAVLYCFLNQEVRTEISRKWYGHDPELLP
                **************************************************

Pituitary       ARRTCTEWTTPPRSRVKVLTSEC
Renal           ARRTCTEWTTPPRSRVKVLTSEC
                ***********************
```

* = identity between the anterior pituitary and renal medulla GHRH-R protein sequence

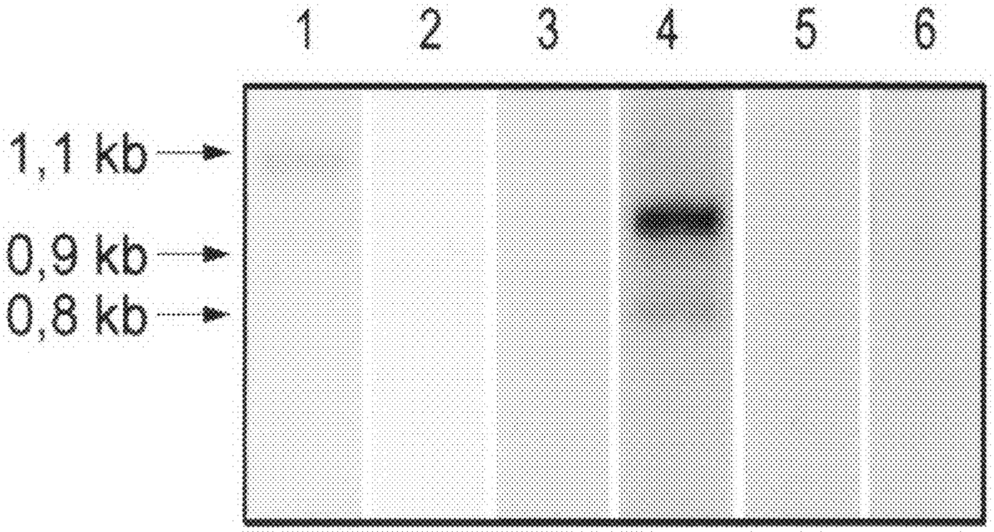


Figure 16A

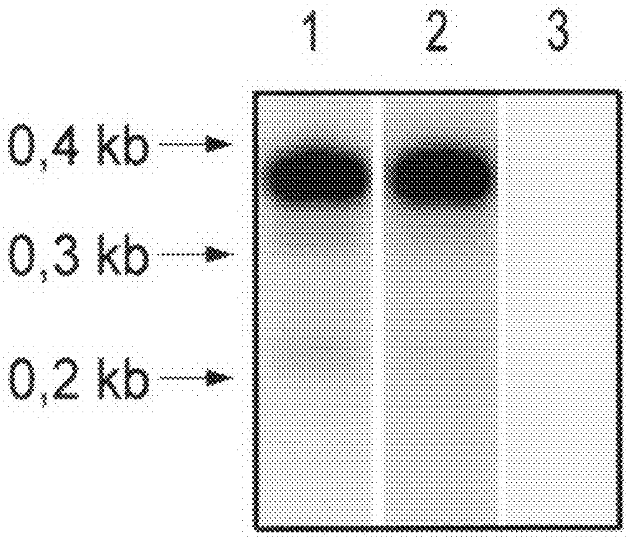


Figure 16B

Figure 17

A (400 aa)

aaacacacctgaaactactgtgagcaggtattgggccac[illegible]CACCCAGAATGTGACTTCATCACCCAGCT
GAGAGAGGATGAGAGTGCCTGTCTACAAGCAGCAGAGGAG**A**TGCCCAACACCACCCTGGGCTGCCCTGCGA
CCTGGGATGGGCTGCTGTGCTGGCCAACGGCAGGCTCTGGCGAGTGGGTCACCCTCCCCTGCCCGGATTTC
TTCTCTCACTTCAGCTCAGAGTCAGGGGCTGTGAAACGGGATTGTACTATCACTGGCTGGTCTGAGCCCTT
TCCACCTTACCCTGTGGCCTGCCCTGTGCCTCTGGAGCTGCTGGCTGAGGAGGAATCTTACTTCTCCACAG
TGAAGATTATCTACACCGTGGGCCATAGCATCTCTATTGTAGCCCTCTTCGTGGCCATCACCATCCTGGTT
GCTCTCAGGAGGCTCCACTGCCCCCGGAACTACGTCCACACCCAGCTGTTCACCACTTTTATCCTCAAGGC
GGGAGCTGTGTTCCTGAAGGATGCTGCCCTTTTCCACAGCGACGACACTGACCACTGCAGCTTCTCCACTG
TTCTATGCAAGGTCTCTGTGGCCGCCTCCCATTTCGCCACCATGACCAACTTCAGCTGGCTGTTGGCAGAA
GCCGTCTACCTGAACTGCCTCCTGGCCTCCACCTCCCCCAGCTCAAGGAGAGCCTTCTGGTGGCTGGTTCT
CGCTGGCTGGGGGCTGCCCGTGCTCTTCACTGGCACGTGGGTGAGCTGCAAACTGGCCTTCGAGGACATCG
CGTGCTGGGACCTGGACGACACCTCCCCCTACTGGTGGATCATCAAAGGGCCCATTGTCCTCTCGGTCGGG
GTGAACTTTGGGCTTTTTCTCAATATTATCCGCATCCTGGTGAGGAAACTGGAGCCAGCTCAGGGCAGCCT
CCATACCCAGTCTCAGTATTGGCGTCTCTCCAAGTCGACACTTTTCCTGATCCCACTCTTTGGAATTCACT
ACATCATCTTCAACTTCCTGCCAGACAATGCTGGCCTGGGCATCCGCCTCCCCCTGGAGCTGGGACTGGGT
TCCTTCCAGGGCTTCATTGTTGCCATCCTCTACTGCTTCCTCAACCAAGAGGTGAGGACTGAGATCTCACG
GAAGTGGCATGGCCATGACCCTGAGCTTCTGCCAGCCTGGAGGACCCGTGCTAAGTGGACCACGCCTTCCC
GCTCGGCGGCAAAGGTGCTGACATCTATGTGCTAGgctgcctcatcacgccactggagtccacacttgaat
ttgggcagctaccacgggtctgccatgctctggaggagcaaggggggccacatccccacccagctgttacc
cagcccggggcaggtgcagcccttcctccctgtctctgc**g**tctgactctctttgaggtccctgtatgtct
acctctgacttctgtggtccctctgtgtctgctctcatccattcccctactggggc**a**tggggctctagcc
caaggctcagaggagcc[illegible]cctgtaaatgaaaaaaaaaaaaa

nucleotides varying in comparison to the genomic sequence			
	Frequency of occurence	Nucleotide in the human genomic sequence	Impact on the protein sequence
C	6/7	A	None
A	6/6	C	None

nucleotides varying in 5′ UTR of the 400 aa protein	
Nucleotide in the 5′ UTR human renal sequence	Frequency of occurence
AAACACACCTGAAACTACTGTGAGCAG	8/10
CCTTCCTTCCATCTGAACATCTACCAAACACGCCTGAAACTACTGTGAGCAGCAG	2/10

nucleotides varying in human renal ORF of the 400 aa protein			Impact on the protein sequence
	Frequency of occurence	Nucleotide substitution	
A	2/12	G	None

Figure 17

B (416 aa)

```
cttccttccttccttccttcccatctgaacatctaccaaacacacctgaaactactgtgagcaggtct
ttgcctaggattagggcacagatatgaatcaggccttgtccctgttctcaggtc[illegible]TGGACCACAGAGCC
CAGAAAGACACCCAAATGGCTTGGCTCATCCTGTTCACTGTTTCCAGCAGCCTCACCCCTCGGATTATTGG
GACAGCCCTGCACCTGGGCTGAGTCTCTGCTGCTCCTGGCTCTCTATCCAGGCTGCCCTGCGACCTGGGAT
GGGCTGCCTGTGCTGGCCAACGGCAGGCTCTGGCGAGTGGGTCACCCTCCCCTGCCCGGATTTCTTCTCTCA
CTTCAGCTCAGAGTCAGGGGCTGTGAAACGGGATTGTACTATCACTGGCTGGTCTGAGCCCTTTCCACCTT
ACCCTGTGGCCTGCCCTGTGCCTCTGGAGCTGCTGGCTGAGGAGGAATCTTACTTCTCCACAGTGAAGATT
ATCTACACCGTGGGCCATAGCATCTCTATTGTAGCCCTCTTCGTGGCCATCACCATCCTGGTTGCTCTCAG
GAGGCTCCACTGCCCCCGGAACTACGTCCACACCCAGCTGTTCACCACTTTTATCCTCAAGGCGGGAGCTG
TGTTCCTGAAGGATGCTGCCCTTTTCCACAGCGACGACACTGACCACTGCAGCTTCTCCACTGTTCTATGC
AAGGTCTCTGTGGCCGCCTCCCATTTCGCCACCATGACCAACTTCAGCTGGCTGTTGGCAGAAGCCGTCTA
CCTGAACTGCCTCCTGGCCTCCACCTCCCCCAGCTCAAGGAGAGCCTTCTGGTGGCTGGTTCTCGCTGGCT
GGGGCTGCCCGTGCTCTTCACTGGCACGTGGGTGAGCTGCAAACTGGCCTTCGAGGACATCGCGTGCTGG
GACCTGGACGACACCTCCCCCTACTGGTGGATCATCAAAGGGCCCATTGTCCTCTCGGTCGGGGTGAACTT
TGGCTTTTTTCTCAATATTATCCGCATCCTGGTGAGGAAACTGGAGCCAGCTCAGGGCAGCCTCCATACCC
AGTCTCAGTATTGGCGTCTCTCCAAGTCGACACTTTTCCTGATCCCACTCTTTGGAATTCACTACATCATC
TTCAACTTCCTGCCAGACAATGCTGGCCTGGGCATCCGCCTCCCCCTGGAGCTGGGACTGGGTTCCTTCCA
GGGCTTCATTGTTGCCATCCTCTACTGCTTCCTCAACCAAGAGGTGAGGACTGAGATCTCACGGAAGTGGC
ATGGCCATGACCCTGAGCTTCTGCCAGCCTGGAGGACCCCGTGCTAAGTGGACCACGCCTTCCCGCTCGGCG
GCAAAGGTGCTGACATCTATGTCCTAGgctgcctcatcacgccactggagtccacacttgaatttgggcag
ctaccacgggtctgccatgctctggaggagcaagggggccacatccccaccccagctgttacccagcccgg
ggcaggtgcagcccttcctccctgtctctgcgtctgactctctttgaggtccctgcatgtctacctctga
cttctgtggtccctctgtgtctgctctcatccattcctcttactggggcatggggctctagcccaaggctc
agaggagcc[illegible]cctgtaaatgaaaaaaaaaaaaaa
```

nucleotides varying in comparison to the genomic sequence			Impact on the protein sequence
C	6/7	A	None
A	6/6	C	None

nucleotides varying in human renal ORF of the 416 aa protein			Impact on the protein sequence
	Frequency of occurence	Nucleotide substitution	
T	4/16	C	None

Figure 18

A (400 aa)

```
  40 atgcacccagaatgtgacttcatcacccagctgagagaggatgag
     M  H  P  E  C  D  F  I  T  Q  L  R  E  D  E
  85 agtgcctgtctacaagcagcagaggagatgcccaacaccaccctg
     S  A  C  L  Q  A  A  E  E  M  P  N  T  T  L
 130 ggctgccctgcgacctgggatgggctgctgtgctggccaacggca
     G  C  P  A  T  W  D  G  L  L  C  W  P  T  A
 175 ggctctggcgagtgggtcaccctcccctgcccggatttcttctct
     G  S  G  E  W  V  T  L  P  C  P  D  F  F  S
 220 cacttcagctcagagtcaggggctgtgaaacgggattgtactatc
     H  F  S  S  E  S  G  A  V  K  R  D  C  T  I
 265 actggctggtctgagccctttccaccttaccctgtggcctgccct
     T  G  W  S  E  P  F  P  P  Y  P  V  A  C  P
 310 gtgcctctggagctgctggctgaggaggaatcttacttctccaca
     V  P  L  E  L  L  A  E  E  E  S  Y  F  S  T
 355 gtgaagattatctacaccgtgggccatagcatctctattgtagcc
     V  K  I  I  Y  T  V  G  H  S  I  S  I  V  A
 400 ctcttcgtggccatcaccatcctggttgctctcaggaggctccac
     L  F  V  A  I  T  I  L  V  A  L  R  R  L  H
 445 tgcccccggaactacgtccacacccagctgttcaccacttttatc
     C  P  R  N  Y  V  H  T  Q  L  F  T  T  F  I
 490 ctcaaggcgggagctgtgttcctgaaggatgctgccctttttccac
     L  K  A  G  A  V  F  L  K  D  A  A  L  F  H
 535 agcgacgacactgaccactgcagcttctccactgttctatgcaag
     S  D  D  T  D  H  C  S  F  S  T  V  L  C  K
 580 gtctctgtggccgcctcccatttcgccaccatgaccaacttcagc
     V  S  V  A  A  S  H  F  A  T  M  T  N  F  S
 625 tggctgttggcagaagccgtctacctgaactgcctcctggcctcc
     W  L  L  A  E  A  V  Y  L  N  C  L  L  A  S
 670 acctcccccagctcaaggagagccttctggtggctggttctcgct
     T  S  P  S  S  R  R  A  F  W  W  L  V  L  A
 715 ggctgggggctgcccgtgctcttcactggcacgtgggtgagctgc
     G  W  G  L  P  V  L  F  T  G  T  W  V  S  C
 760 aaactggccttcgaggacatcgcgtgctgggacctggacgacacc
     K  L  A  F  E  D  I  A  C  W  D  L  D  D  T
 805 tcccccctactggtggatcatcaaagggcccattgtcctctcggtc
     S  P  Y  W  W  I  I  K  G  P  I  V  L  S  V
 850 ggggtgaactttgggctttttctcaatattatccgcatcctggtg
     G  V  N  F  G  L  F  L  N  I  I  R  I  L  V
 895 aggaaactggagccagctcagggcagcctccatacccagtctcag
     R  K  L  E  P  A  Q  G  S  L  H  T  Q  S  Q
 940 tattggcgtctctccaagtcgacacttttcctgatcccactcttt
     Y  W  R  L  S  K  S  T  L  F  L  I  P  L  F
 985 ggaattcactacatcatcttcaacttcctgccagacaatgctggc
     G  I  H  Y  I  I  F  N  F  L  P  D  N  A  G
1030 ctgggcatccgcctcccccctggagctgggactgggttccttccag
     L  G  I  R  L  P  L  E  L  G  L  G  S  F  Q
1075 ggcttcattgttgccatcctctactgcttcctcaaccaagaggtg
     G  F  I  V  A  I  L  Y  C  F  L  N  Q  E  V
1120 aggactgagatctcacggaagtggcatggccatgaccctgagctt
     R  T  E  I  S  R  K  W  H  G  H  D  P  E  L
1165 ctgccagcctggaggacccgtgctaagtggaccacgccttcccgc
     L  P  A  W  R  T  R  A  K  W  T  T  P  S  R
1210 tcggcggcaaaggtgctgacatctatgtgctag 1242
     S  A  A  K  V  L  T  S  M  C  *
```

Figure 18

B (416 aa)

```
 126 atgtggaccacagagcccagaaagacacccaaatggcttggctca
     M  W  T  T  E  P  R  K  T  P  K  W  L  G  S
 171 tcctgttcactgtttccagcagcctcacccctcggattattggga
     S  C  S  L  F  P  A  A  S  P  L  G  L  L  G
 216 cagccctgcacctgggctgagtctctgctgctcctggctctctat
     Q  P  C  T  W  A  E  S  L  L  L  L  A  L  Y
 261 ccaggctgccctgcgacctgggatgggctgctgtgctggccaacg
     P  G  C  P  A  T  W  D  G  L  L  C  W  P  T
 306 gcaggctctggcgagtgggtcaccctcccctgcccggatttcttc
     A  G  S  G  E  W  V  T  L  P  C  P  D  F  F
 351 tctcacttcagctcagagtcaggggctgtgaaacgggattgtact
     S  H  F  S  S  E  S  G  A  V  K  R  D  C  T
 396 atcactggctggtctgagccctttccaccttaccctgtggcctgc
     I  T  G  W  S  E  P  F  P  P  Y  P  V  A  C
 441 cctgtgcctctggagctgctggctgaggaggaatcttacttctcc
     P  V  P  L  E  L  L  A  E  E  E  S  Y  F  S
 486 acagtgaagattatctacaccgtgggccatagcatctctattgta
     T  V  K  I  I  Y  T  V  G  H  S  I  S  I  V
 531 gccctcttcgtggccatcaccatcctggttgctctcaggaggctc
     A  L  F  V  A  I  T  I  L  V  A  L  R  R  L
 576 cactgcccccggaactacgtccacacccagctgttcaccactttt
     H  C  P  R  N  Y  V  H  T  Q  L  F  T  T  F
 621 atcctcaaggcgggagctgtgttcctgaaggatgctgcccttttc
     I  L  K  A  G  A  V  F  L  K  D  A  A  L  F
 666 cacagcgacgacactgaccactgcagcttctccactgttctatgc
     H  S  D  D  T  D  H  C  S  F  S  T  V  L  C
 711 aaggtctctgtggccgcctcccatttcgccaccatgaccaacttc
     K  V  S  V  A  A  S  H  F  A  T  M  T  N  F
 756 agctggctgttggcagaagccgtctacctgaactgcctcctggcc
     S  W  L  L  A  E  A  V  Y  L  N  C  L  L  A
 801 tccacctcccccagctcaaggagagccttctggtggctggttctc
     S  T  S  P  S  S  R  R  A  F  W  W  L  V  L
 846 gctggctgggggctgcccgtgctcttcactggcacgtgggtgagc
     A  G  W  G  L  P  V  L  F  T  G  T  W  V  S
 891 tgcaaactggccttcgaggacatcgcgtgctgggacctggacgac
     C  K  L  A  F  E  D  I  A  C  W  D  L  D  D
 936 acctcccccctactggtggatcatcaaagggcccattgtcctctcg
     T  S  P  Y  W  W  I  I  K  G  P  I  V  L  S
 981 gtcggggtgaactttgggctttttctcaatattatccgcatcctg
     V  G  V  N  F  G  L  F  L  N  I  I  R  I  L
1026 gtgaggaaactggagccagctcagggcagcctccatacccagtct
     V  R  K  L  E  P  A  Q  G  S  L  H  T  Q  S
1071 cagtattggcgtctctccaagtcgacacttttcctgatcccactc
     Q  Y  W  R  L  S  K  S  T  L  F  L  I  P  L
1116 tttggaattcactacatcatcttcaacttcctgccagacaatgct
     F  G  I  H  Y  I  I  F  N  F  L  P  D  N  A
1161 ggcctgggcatccgcctcccccctggagctgggactgggttccttc
     G  L  G  I  R  L  P  L  E  L  G  L  G  S  F
1206 cagggcttcattgttgccatcctctactgcttcctcaaccaagag
     Q  G  F  I  V  A  I  L  Y  C  F  L  N  Q  E
1251 gtgaggactgagatctcacggaagtggcatggccatgaccctgag
     V  R  T  E  I  S  R  K  W  H  G  H  D  P  E
1296 cttctgccagcctggaggacccgtgctaagtggaccacgccttcc
     L  L  P  A  W  R  T  R  A  K  W  T  T  P  S
1341 cgctcggcggcaaaggtgctgacatctatgtgctag 1376
     R  S  A  A  K  V  L  T  S  M  C  *
```

Figure 19

A. (400 aa)

```
CLUSTAL W (1.81) multiple sequence alignment


Renal_medulla_400_aa      -----------------------MHPECDFITQLREDESACLQAAEEMPNTTLGCPATWD
Anterior_pituitary        MDRRMWGAHVFCVLSPLPTVLGHMHPECDFITQLREDESACLQAAEEMPNTTLGCPATWD
                                                 *************************************

Renal_medulla_400_aa      GLLCWPTAGSGEWVTLPCPDFFSHFSSESGAVKRDCTITGWSEPFPPYPVACPVPLELLA
Anterior_pituitary        GLLCWPTAGSGEWVTLPCPDFFSHFSSESGAVKRDCTITGWSEPFPPYPVACPVPLELLA
                          ************************************************************

Renal_medulla_400_aa      EEESYFSTVKIIYTVGHSISIVALFVAITILVALRRLHCPRNYVHTQLFTTFILKAGAVF
Anterior_pituitary        EEESYFSTVKIIYTVGHSISIVALFVAITILVALRRLHCPRNYVHTQLFTTFILKAGAVF
                          ************************************************************

Renal_medulla_400_aa      LKDAALFHSDDTDHCSFSTVLCKVSVAASHFATMTNFSWLLAEAVYLNCLLASTSPSSRR
Anterior_pituitary        LKDAALFHSDDTDHCSFSTVLCKVSVAASHFATMTNFSWLLAEAVYLNCLLASTSPSSRR
                          ************************************************************

Renal_medulla_400_aa      AFWWLVLAGWGLPVLFTGTWVSCKLAFEDIACWDLDDTSPYWWIIKGPIVLSVGVNFGLF
Anterior_pituitary        AFWWLVLAGWGLPVLFTGTWVSCKLAFEDIACWDLDDTSPYWWIIKGPIVLSVGVNFGLF
                          ************************************************************

Renal_medulla_400_aa      LNIIRILVRKLEPAQGSLHTQSQYWRLSKSTLFLIPLFGIHYIIFNFLPDNAGLGIRLPL
Anterior_pituitary        LNIIRILVRKLEPAQGSLHTQSQYWRLSKSTLFLIPLFGIHYIIFNFLPDNAGLGIRLPL
                          ************************************************************

Renal_medulla_400_aa      ELGLGSFQGFIVAILYCFLNQEVRTEISRKWHGHDPELLPAWRTRAKWTTPSRSAAKVLT
Anterior_pituitary        ELGLGSFQGFIVAILYCFLNQEVRTEISRKWHGHDPELLPAWRTRAKWTTPSRSAAKVLT
                          ************************************************************

Renal_medulla_400_aa      SMC
Anterior_pituitary        SMC
                          ***
```

* = identity between the anterior pituitary and renal medulla GHRH-R protein sequence

Figure 19

B. (416 aa)

```
CLUSTAL W (1.81) multiple sequence alignment


Renal_medulla_416_aa      ----MWTTEPRKTPKWLGSSCSLFPAASPLGLLG---QPCTWAESLLLLALYPGCPATWD
Anterior_pituitary        MDRRMWGAHVFCVLSPLPTVLGHMHPECDFITQLREDESACLQAAEEMPNTTLGCPATWD
                              **          *                                    *******

Renal_medulla_416_aa      GLLCWPTAGSGEWVTLPCPDFFSHFSSESGAVKRDCTITGWSEPFPPYPVACPVPLELLA
Anterior_pituitary        GLLCWPTAGSGEWVTLPCPDFFSHFSSESGAVKRDCTITGWSEPFPPYPVACPVPLELLA
                          ************************************************************

Renal_medulla_416_aa      EEESYFSTVKIIYTVGHSISIVALFVAITILVALRRLHCPRNYVHTQLFTTFILKAGAVF
Anterior_pituitary        EEESYFSTVKIIYTVGHSISIVALFVAITILVALRRLHCPRNYVHTQLFTTFILKAGAVF
                          ************************************************************

Renal_medulla_416_aa      LKDAALFHSDDTDHCSFSTVLCKVSVAASHFATMTNFSWLLAEAVYLNCLLASTSPSSRR
Anterior_pituitary        LKDAALFHSDDTDHCSFSTVLCKVSVAASHFATMTNFSWLLAEAVYLNCLLASTSPSSRR
                          ************************************************************

Renal_medulla_416_aa      AFWWLVLAGWGLPVLFTGTWVSCKLAFEDIACWDLDDTSPYWWIIKGPIVLSVGVNFGLF
Anterior_pituitary        AFWWLVLAGWGLPVLFTGTWVSCKLAFEDIACWDLDDTSPYWWIIKGPIVLSVGVNFGLF
                          ************************************************************

Renal_medulla_416_aa      LNIIRILVRKLEPAQGSLHTQSQYWRLSKSTLFLIPLFGIHYIIFNFLPDNAGLGIRLPL
Anterior_pituitary        LNIIRILVRKLEPAQGSLHTQSQYWRLSKSTLFLIPLFGIHYIIFNFLPDNAGLGIRLPL
                          ************************************************************

Renal_medulla_416_aa      ELGLGSFQGFIVAILYCFLNQEVRTEISRKWHGHDPELLPAWRTRAKWTTPSRSAAKVLT
Anterior_pituitary        ELGLGSFQGFIVAILYCFLNQEVRTEISRKWHGHDPELLPAWRTRAKWTTPSRSAAKVLT
                          ************************************************************

Renal_medulla_416_aa      SMC
Anterior_pituitary        SMC
                          ***
```

* = identity between the anterior pituitary and renal medulla GHRH-R protein sequence

RENOPROTECTION BY GROWTH HORMONE-RELEASING HORMONE AND AGONISTS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This patent application claims the benefit of U.S. Provisional patent application Ser. No. 60/960,477 filed Oct. 1, 2007 and Ser. No. 61/006,057 filed Dec. 17, 2007 the disclosure of which are herein incorporated by reference in their entirety.

FIELD OF INVENTION

[0002] The present invention relates to the field of renoprotection by growth hormone-releasing hormone and agonists.

BACKGROUND OF THE INVENTION

[0003] The pituitary growth hormone-releasing hormone receptor (GHRH-R) has been cloned in several mammalian species,[1-4] including normal human pituitary[2, 5, 6] and adenomas.[5-7] More recently, GHRH-R was reported in avian[8] and fish pituitary.[9] The rat pituitary contains a major GHRH-R mRNA transcript (2.5 kb) and a less abundant one (4 kb; ≈20% of the 2.5-kb in 2-month-old rats).[2, 10] While the 2.5-kb transcript generates the 423 amino acid functional GHRH-R,[11] the role and structure of the 4-kb transcript remain to be elucidated. The 47-kDa-encoded rat protein belongs to the subfamily B-III of G protein-coupled receptors, which also include receptors for VIP, secretin, glucagon, GIP, PTH, calcitonin, CRF and PACAP.[2] In somatotrophs, the specific binding of hypothalamic GHRH to functional plasma membrane receptor represents the primary event leading to GH secretion[12-13] and synthesis[12] mainly through an adenylate cyclase/cAMP/protein kinase (PK) A pathway[14-17] and possibly a PKC pathway.[18] GHRH-mediated GHRH-R activation is also involved in somatotroph proliferation and differentiation via PKA[2, 19-22] and mitogen-activated protein (MAP) kinase pathways.[23-24]

[0004] Apart from the anterior pituitary, a GHRH-GHRH-R system has been identified in rat brain, spleen and thymus, ovary, placenta, testis and renal medulla. Intrasuprachiasmatic/medial preoptic area administration of GHRH stimulates dietary protein intake in free-feeding rats[25] while it promotes sleep in the intrapreoptic region.[26] *In rat spleen and thymus, a functional GHRH-GH axis was shown to mediate lymphocyte proliferation through a GHRH-induced GH mechanism.*[27] In human and rat reproductive systems, the presence of GHRH-R mRNA[2] and immunoreactivity[28] has been reported as well as GHRH-mediated effects on regulation of sex steroid levels,[29] granulosa cell differentiation,[30] placental growth,[31] and gonadotropin stimulation of testosterone.[32]

[0005] A functional GHRH-R has been identified in the rat renal medulla.[33, 34] Boulanger et al. demonstrated the presence of specific, reversible and saturable binding for [^{125}]-Tyr^{10}]hGHRH(1-44)NH$_2$ in this tissue.[34] Moreover, stimulation of semi-purified Henle's loop (HL) cells with GHRH was shown to mediate GHRH-R internalization and regulation of its expression.[33] The highest level of renal GHRH-R mRNA was localized in HL by ribonuclease protection assay and in situ hybridization.[33] Its localization in HL and the tissue-selective regulation of pituitary and renal GHRH-R mRNA levels and its regulation during development and aging may suggests roles of GHRH-R in the renal medulla.[33]

[0006] The present description refers to a number of documents, the content of which is herein incorporated by reference in their entirety.

SUMMARY OF THE INVENTION

[0007] In one aspect thereof, the present invention relates to a method for protecting and/or treating a mammal against oxidative renal damage. The method may comprise the step of administering an effective amount of a ligand to (of the) GHRH renal receptor to the mammal.

[0008] In another aspect, the present invention relates to a method for preventing (lowering, inhibiting) the death of kidney cells (and/or loss of kidney cell function) due to oxidative stress in a mammal in need thereof. The method may comprise administering to the mammal a ligand to GHRH renal receptor.

[0009] In a further aspect, the present invention relates to a method of promoting regeneration of kidney cells and/or function in a mammal in need thereof. The method may comprise administering a ligand to the GHRH renal receptor the mammal.

[0010] In yet a further aspect thereof, the present invention relates to the new polypeptidic sequence of rat and/or human GHRH receptors, antibody that may bind same, nucleic acids that may encode same, a cell expressing same and/or vectors that may comprise the nucleic acid of the present invention.

[0011] In another aspect, the present invention relates to an assay for identifying a ligand capable of specific binding to the new rat and/or to the new human GHRH receptors and not to pituitary GHRH receptor. This assay may comprise contacting a test ligand with the polypeptide, measuring binding of the test ligand to the polypeptide and/or determining the identity of the test ligand.

[0012] In a further aspect, the present invention relates to a method for the diagnosis of renal oxidative stress and/or damage and diagnostic kits.

[0013] Further scope, applicability and advantages of the present invention will become apparent from the non-restrictive detailed description given hereinafter. It should be understood, however, that this detailed description, while indicating exemplary embodiments of the invention is given by way of example only, with reference to the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] In the appended drawings which illustrates non-limitative exemplary embodiments of the present invention,

[0015] FIG. **1** GHRH-R mRNA levels in the renal medulla and purified thin and thick HL cells from 2-month-old healthy rats. Five (thin HL) and 20 μg (thick HL and medulla) of total RNA were analyzed by RPA. Results were expressed as per 20 μg of total RNA, in percentage of relative density to that obtained in 20 μg total RNA samples from the medulla. Results represent the mean±SEM of samples analyzed in duplicate from 2 independent RPA experiments and were normalized with both GAPDH and the cRNA external standard. $**P<0.01$ when compared to GHRH-R mRNA levels in total medulla (Dunnett's test);

[0016] FIG. **2** Immunocytochemical localization of the GHRH-R in purified thin limb cells from 2-month-old healthy rats. Co-localization of GHRH-R immunofluorescence (b, e) was assessed in renal cells from 2-month-old male rats using an anti-aquaporin-1 antibody, as a marker of descending thin limb cells (a) and an anti-ClC-K antibody, as a marker of ascending thin limb cells (d). Immunolabeling was specific and no labeling was observed when substituting the anti-GHRH-R(392-404) Ab for normal IgGs (data not shown). Nuclei were labeled with DAPI. Results are representative of three independent experiments;

[0017] FIG. **3** Visualization of immunoreactive GHRH and ClC-K1 chloride channel and PCR amplification of pre-proGHRH in purified thin limb cells from 2 month-old healthy rats. A) Labeling of GHRH (a, b) and the ClC-K1 chloride channel (c, d) was performed in purified thin limb cells using an anti-rat GHRH(1-43)OH Ab and an anti-ClC-K Ab. Overlay of GHRH and ClC-K immunofluorescence is shown in (f). The specificity of labeling was assessed by substituting the anti-rat GHRH(1-43)OH Ab for normal IgGs (e). B) Representative agarose gel electrophoresis of pre-proGHRH and GAPDH PCR products and molecular weight markers.

[0018] FIG. **4** Effect of a 2-, 7- or 14-day 8%-NaCl dietary intake on medullary GHRH-R mRNA levels from 2-months-old rats. A) Autoradiographic representation of GHRH-R mRNA, GAPDH mRNA and RPR-64 Msc I cRNA external standard (40 pg) signals analyzed by RPA, from rats fed 8%- or 0.3%-NaCl (control) diet. B) GHRH-R mRNA levels expressed per 20 μg total RNA. For the 2-day experiment, 5-6 individual rats were used in each group for both RPA and statistical analysis, while for the 7- and 14-day experiment, 7-8 individual rats were used. Results are expressed in percentage of relative density to that obtained in the medulla from control rats and represent the mean±SEM of individual samples from each group, analyzed in triplicate twice and normalized with GAPDH and the cRNA external standard. $*P<0.05$ and $**P<0.01$ when compared to GHRH-R mRNA levels in the medulla from control rats (Student's t test);

[0019] FIG. **5** Effects of a 2-, 7- or 14-day 8%-NaCl dietary intake and a 3-day water deprivation on anterior pituitary GHRH-R mRNA levels from 2-months-old rats. A-D) GHRH-R mRNA levels analyzed by Northern blotting and expressed per 12 μg total RNA. For the 2- (A), 7- (B) and 14-day (C) 8%-NaCl experiment, 7-8 individual rats were used in each group for both Northern blotting and statistical analysis, while for the 3-day water deprivation (D), 3 (controls) and 7 (deprived) individual rats were used. Results are expressed in percentage of relative density to that obtained in the pituitary from control rats and represent the mean±SEM of individual samples from each group, analyzed in duplicate and normalized with normalized with rRNA 28S. $*P<0.05$ and $***P<0.001$ when compared to GHRH-R mRNA levels in the pituitary from control rats (Student's t test);

[0020] FIG. **6** Basal and GHRH-stimulated cAMP levels in semi-purified thin limb cells from 2-month-old rats, following a 2-, 7- or 14-day 8%-NaCl dietary intake. Basal and net GHRH-stimulated cAMP levels were quantified by EIA (fmol/μg prot) in freshly dispersed semi-purified thin limb cells of rats fed 2- (A), 7- (B) or 14- (C) days a 8%- or 0.3%-NaCl (control) diet. Results are expressed in percentage of control values both for basal and stimulated cAMP levels. Cells from 4 individual rats were used in each diet and control group. $*P<0.05$ and $**P<0.01$ when compared to cAMP levels in semi-purified thin limb cells from control rats (Student's t test);

[0021] FIG. **7** Effect of a GHRH in vivo sc administration of GHRH in 2 month-old rats fed a 8%- or 0.3%-NaCl diet on anti-BrdU labeling. BrdU was injected ip 2 h prior sacrifice (100 mg/hg BW). Rats were fed a 8%- or 0.3%-NaCl (control) diet and concurrently injected with rGHRH(1-29)NH_2 (1 mg/kg BW). Purified thin limb cells were cultured 16 h on coverslips and processed for immunocytochemistry. A) Increased number of cells exhibiting specific anti-BrdU labeling, colocalizing either with DAPI (nuclear) or Mitotracker red CMXRos (mitochondrial) and B) increased anti-BrdU total fluorescence intensity in nuclear or mitochondrial compartment were expressed in percentage of control values (0.3%-NaCL salt diet, GHRH vehicle injection). $*P<0.05$ when compared to levels in purified thin limb cells from control rats (Dunnett's t test);

[0022] FIG. **8** Effect of a GHRH in vivo sc administration in 2 months-old rats on the regulation of GHRH-R and ClC-K1 mRNA levels in purified thin limb cells. Two-month-old healthy male Sprague Dawley rats, received a subcutaneous administration of rGHRH(1-29)NH_2 (1 mg/kg BW/day) or the saline vehicle for 2 days. (A) GHRH-R and ClC-K1 (B) mRNA levels were analyzed by real-time RT-PCR. Eight animals were used in control and treatment. Group 1=3 rats. Group 2=5 rats. $*P<0.05$ and $**P<0.01$ when compared to levels in purified thin limb cells from control rats (Dunnett's t test) (C) Regulation of GHRH-R mRNA levels in thin limb cells of 2-month-old healthy male Sprague Dawley rats was also studied following a subcutaneous administration of rGHRH(1-29)NH2 (0.5 mg/kg BW/day) or the saline vehicle, for 2 days;

[0023] FIG. **9** rGHRH(1-29)NH_2-induced cell proliferation in semi-purified thin limb cells. Proliferation was assessed after a 60-h cell culture period, using a CellTiter 96R Aqueous one solution cell proliferation assay. Results represent the mean±SEM of 2 independent experiments performed in duplicate. $*P<0.05$, $**P<0.01$ when compared to control levels (Dunnett's t test);

[0024] FIG. **10** RT-PCR products from rat and porcine renal medulla and anterior pituitary obtained with a panel of primers of the pituitary GHRH-R. A) Rat and B) porcine total RNA was used. Lanes 1, 2: sense and antisense 5' end primers, lanes 3, 4: sense and antisense middle portion and lanes 5, 6: sense and antisense 3' end primers, respectively;

[0025] FIG. **11** GHRH-R 5'- and 3'-RACE products from rat renal medulla and anterior pituitary Poly(A)$^+$RNA. A, Renal medulla (lane 1), anterior pituitary (lane 2) and liver (lane 3, negative control) 5' cDNA products were obtained using the primer pair NUP/Ex-9/10P (Table 2). Each product from the renal medulla and anterior pituitary was isolated by gel extraction, (renal medulla: lanes 4-6; pituitary: lane 7). B, Renal medulla (lane 1), anterior pituitary (lane 2) and liver (lane 3) 3' cDNA products were obtained using the primer pair NUP/N-Ex7/8 (Table 2). Each product from the renal medulla and anterior pituitary was isolated by gel extraction, (renal medulla: lanes 4-5; pituitary: lanes 6-7). Data are representative of three independent experiments;

[0026] FIG. **12** Schematic genomic organization of rat GHRH-Rs. Exons organization and genomic location of anterior pituitary and renal medulla GHRH-R on chromosome 4. Numbers correspond to GHRH-R exons. The first and last nucleotides were at position 18576 and 51026 on AC091710 sequence, respectively, while those of the pituitary GHRH-R were in position 38055 and 51026;

[0027] FIG. **13** is the nucleic acid sequence of the rat renal medulla GHRH receptor;

[0028] FIG. **14** is the deduced protein sequence in the rat renal medulla: 371 aa, the 52 first N-terminal amino acid segment is absent;

[0029] FIG. **15** illustrates a comparison of the rat GHRH-R protein sequence between anterior pituitary and the renal medulla;

[0030] FIG. **16** RT-PCR validation of renal medulla GHRH-R variants in rat thin limb Henle's loop cells and anterior pituitary total RNA. A, tlHL cells (lanes 1, 4), anterior pituitary (lanes 2, 5) and liver (lanes 3, 6) GHRH-R cDNA products using primers pairs Ex-1M/Ex-11M (lanes 1-3) and Ex-2M/Ex-11M (lanes 4,-7). B, tlHL cells (lane 1), anterior pituitary (lane 2) and liver (lane 3) GHRH-R cDNA products using the primer pair Ex-7M/Ex-11M (exon 6 and 10 in anterior pituitary). Data are representative of two independent experiments;

[0031] FIG. **17** is the nucleic acid sequences encoding a 400 amino acids protein (A) or encoding a 416 amino acids protein (B) of human renal medulla GHRH receptor;

[0032] FIG. **18** is the deduced protein sequences of human renal medulla GHRH receptor of 400 amino acids (A) or 416 amino acids (B); and

[0033] FIG. **19** illustrates a comparison of human GHRH-R protein sequence of 400 amino acids (A) or 416 amino acids (B) between anterior pituitary and the renal medulla.

DETAILED DESCRIPTION

[0034] In order to provide a clear and consistent understanding of the terms used in the present disclosure, a number of definitions are provided below. Moreover, unless defined otherwise, all technical and scientific terms as used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention pertains.

[0035] In one aspect thereof, the present invention relates to a method for protecting (and/or treating) a mammal against oxidative renal damage. The method may comprise the step of administering an effective amount of a ligand to GHRH renal receptor to the mammal.

[0036] Oxidative stress occurs inside cells or tissues when production of oxygen radicals exceeds their antioxidant capacity. Excess of free radicals may damage essential macromolecules such as, for example, protein, lipids and DNA, leading to abnormal gene expression, disturbance in receptor activity and signaling, apoptosis, immunity perturbation, mutagenesis, and protein or lipofushin deposition. Numerous human diseases involve localized or general oxidative stress. In many serious diseases such as cancer, ocular degeneration (age-related macular degeneration or cataract) and neurodegenerative diseases (ataxia, amyotrophic lateral sclerosis, Alzheimer's disease), oxidative stress is one of the primary disease factor. In various other diseases, oxidative stress occurs secondary to the initial disease and plays an important in role in immune and vascular complications, such as, for example, in AIDS, septic shock, Parkinson's disease, diabetes and renal failure. It is also the case in aging, were accumulation of cellular oxidative stress is considered as a key element in the deterioration of tissues, organs and systems. In an embodiment of the present invention, the oxidative stress may be renal oxidative stress and/or renal oxidative damage. Renal oxidative stress and/or renal oxidative damage may lead to impairment and/or loss of renal function. Renal function is an indication of the state of the kidney and its role in renal physiology. For example, glomerular filtration rate (GFR) may be used to describe the flow rate of filtered fluid through the kidney and assess renal function. Creatinine clearance rate (CCr) is also a marker for renal function and corresponds to the volume of blood plasma that is cleared of creatinine per unit time and is a useful measure for approximating the GFR. Both GFR and CCr may be accurately calculated by comparative measurements of substances in the blood and urine. An exemplary oxidative damage in kidneys may be due to exaggerated renal medullary osmolality.

[0037] In an embodiment of the present invention, oxidative renal damage may affect Henle's loop cells. In another embodiment, the Henle's loop cells affected by oxidative renal damage may more specifically be thin limb Henle's loop cells. In yet a further embodiment, the thin limb Henle's loop cells affected by oxidative renal damage may be ascending thin limb Henle's loop cells.

[0038] According to the present invention, a mammal in need may be identified by various means and methods prior to administration of a GHRH-R ligand, for example, by determining kidney function. The mammal may also be identified by determining the presence of markers associated with oxidative stress (damage) to kidney cells and/or kidney function. In an exemplary embodiment of the present invention, the mammal may be a human being.

[0039] According to the present invention, the term "marker" means any marker of kidney function and/or any stress marker known in the art or as described herein. Stress markers may be oxidatively damaged proteins and/or lipids, active oxygen species (hydroxy radicals, alkoxy radicals, hydroperoxy radicals, peroxy radicals, iron-oxygen complexes, superoxides, hydrogen peroxide, hydroperoxides, singlet oxygen and ozone) or free radicals (lipid radicals and the like). For example, concentrations of two major aldehydic lipid peroxidation (LPO) products, 4-hydroxynonenal (HNE) and malondialdehyde (MDA), and of protein carbonyls may be analyzed as parameters of oxidative stress related to kidney function. Kidney function markers include for example, creatinin, urea, apolipoprotein A-IV. Measurements of these markers (serum measurement, urinary measurement, etc) may be useful to identify a mammal in need (patients) for which the present invention is desirable.

[0040] These markers may be measured in vivo or in bodily fluids such as in urine, serum and/or plasma. Indicators of deterioration of kidney cells such as Henle's loop ascending thin limb cells also include change in urine osmolarity, volume/time urine production and content of urine. As such specific markers alone and/or in combination with indicators of general kidney function may be used to identify the population of patients for which treatment is sought or desirable. Several markers of kidney function are known in the art and markers of oxidative stress (damage to DNA, lipids and/or proteins) to kidney cells have been identified. As such, urinary measurements of these markers may be useful to identify patients for which the present invention is desirable.

[0041] In an exemplary embodiment, the total antioxidant status (TAS) of the mammal may be measured. This assay is based on the capacity of a plasma sample obtained from the mammal to inhibit the formation of 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonate) (ABTS) radicals in the presence of H_2O_2 and metmyoglobine[60]. The percentage of inhibition corresponds to the TAS value expressed in Trolox equivalent. Upon determining the TAS, other plasmatic components are taken into account, namely; concentration of plasma albumin and uric acid. The TAS will thus be determined by the following formula:

$$\text{TAS}=\text{TAS measured}-[(\text{Albumin mmol/l}\times 0.69)+\text{Uric acid mmol/l}\times 1)]$$

[0042] The oxidative capacity of albumin is 0.69 mmol/L Trolox equivalent while the oxidative capacity of uric acid is 1 mmol/L Trolox equivalent. Plasma uric acid levels may be measured by HPLC.

[0043] Oxidative stress to lipids may be determined by evaluating the amount of F2-isoprostane (isomers of prostaglandin F2 (PG F2)) which are formed by the non-enzymatic oxidation of arachnidoic acid under condition of oxidative stress. More particularly, 8-iso-PGF2, the most abundant member of this family is a reliable marker of in vivo oxidative stress to plasma and cellular lipids. To that effect, 8-iso-PGF2 may be extracted from the organic phase of an esterified urine sample (with ester pentafluorbenzyl) and analyzed by gaz chromatography coupled to mass spectroscopy (GC/MS) as per Nourooz-Zadeh et al.

[0044] Oxidative stress to DNA may be determined by measuring the presence of 8-oxo-dGuo in urine. The presence of this product may be detected by HPLC with electrochemical detection as per Arthur et al and Reznick et al.(65, 66)

[0045] A mammal of the invention may suffer or may be susceptible of suffering from a disease that may consist in aging- and frailty-related nephropathy and renal failure, diabetes insipidus, diabetes type I, diabetes II, renal disease glomerulonephritis, bacterial or viral glomerulonephritides, IgA nephropathy, Henoch-Schonlein Purpura, membranoproliferative glomerulonephritis, membranous nephropathy, Sjogren's syndrome, nephrotic syndrome minimal change disease, focal glomerulosclerosis and related disorders, acute renal failure, acute tubulointerstitial nephritis, pyelonephritis, genitourinary (GU) tract inflammatory disease, preclampsia, renal graft rejection, leprosy, reflux nephropathy, nephrolithiasis, genetic renal disease, medullary cystic, medullar sponge, polycystic kidney disease, autosomal dominant polycystic kidney disease, autosomal recessive polycystic kidney disease, tuberous sclerosis, von Hippel-Lindau disease, familial thin-glomerular basement membrane disease, collagen III glomerulopathy, fibronectin glomerulopathy, Alport's syndrome, Fabry's disease, Nail-Patella Syndrome, congenital urologic anomalies, monoclonal gammopathies, multiple myeloma, amyloidosis and related disorders, febrile illness, familial Mediterranean fever, HIV infection, AIDS, inflammatory disease, systemic vasculitides, polyarteritis nodosa, Wegener's granulomatosis, polyarteritis, necrotizing and crescentic glomerulonephritis, polymyositis-dermatomyositis, pancreatitis, rheumatoid arthritis, systemic lupus erythematosus, gout, blood disorders, sickle cell disease, thrombotic thrombocytopenia purpura, hemolytic-uremic syndrome, acute cortical necrosis, renal thromboembolism, trauma and surgery, extensive injury, burns, abdominal and vascular surgery, induction of anesthesia, side effect of drug abuse or use of including those generating renal oxidative stress and toxicity such as antibiotics and cancer chemotherapeutic agents, malignant disease, adenocarcinoma, melanoma, lymphoreticular, multiple myeloma, circulatory disease, myocardial infarction, cardiac failure, peripheral vascular disease, hypertension, coronary heart disease, non-atherosclerotic cardiovascular disease, atherosclerotic cardiovascular disease, skin disease, psoriasis, systemic sclerosis, respiratory disease, chronic obstructive pulmonary disease, obstructive sleep apnea, hypoxia at high altitude or endocrine disease, acromegaly, diabetes mellitus and/or conditions related to antibiotic toxicity, infection, inflammation, ischemia. A mammal of the invention may also be a mammal subjected to chronic hemodialysis.

[0046] By "protecting" a mammal against oxidative renal damage, it is meant a process by which oxidative stress may partially or totally be prevented from damaging renal cells in a mammal. For example, by administering an effective amount of a ligand to GHRH renal receptor in a mammal, renal oxidative damage may partially or totally be prevented. By "treating" a mammal against oxidative renal damage it is meant a process by which the oxidative renal damage is reduced either partially or totally. Treating oxidative renal damage also encompasses a process by which the symptoms of oxidative renal damage in a mammal may not worsen, may remain stable, may be reduced and/or may be completely eliminated.

[0047] As used herein, a "ligand" to the renal GHRH receptor may be native GHRH (SEQ ID NO.:1), a biologically active fragment of GHRH and/or a GHRH agonist thereof. Exemplary embodiments of a GHRH biologically active fragment may include for example, SEQ ID NO.:2 and/or 3. Exemplary embodiment of GHRH agonist may include for example, any one of SEQ ID NO.:4 to 9. Specific embodiments of GHRH agonists may include for example, any one of SEQ ID NO.:4 to 6 wherein Xaa is absent. GHRH agonist of the invention may be capable of activating and/or upregulating renal GHRH receptor.

[0048] In a further exemplary embodiment of the invention, the ligand may be SEQ ID NO.:10, wherein Xaa2 is D-Ala and wherein the remaining amino acid sequence is identical to SEQ ID NO.:1 or 3.

[0049] In an additional exemplary embodiment of the invention, the ligand may be SEQ ID NO.: 10, wherein Xaa10 is D-Tyr and wherein the remaining amino acid sequence is identical to SEQ ID NO.:1 or 3.

[0050] In another exemplary embodiment of the invention, the ligand may be SEQ ID NO.: 10, wherein Xaa15 is D-Ala and wherein the remaining amino acid sequence is identical to SEQ ID NO.:1 or 3.

[0051] In another exemplary embodiment of the invention, the ligand may be SEQ ID NO.:10, wherein Xaa22 is Lys and wherein the remaining amino acid sequence is identical to SEQ ID NO.:1 or 3.

[0052] In yet another exemplary embodiment of the invention, the ligand may be SEQ ID NO.:10, wherein Xaa2 is D-Ala and/or Xaa10 and/or D-Tyr and/or Xaa15 is D-Ala and/or Xaa22 is Lys and wherein the remaining amino acid sequence is identical to SEQ ID NO.:1 or 3.

[0053] In an additional embodiment, the ligand may be SEQ ID NO.:10, wherein Xaa8 is Ala and/or Xaa9 is Ala, and/or Xaa15 is Ala and/or Xaa22 is Ala.

[0054] In yet an additional embodiment, the ligand may be SEQ ID NO.:10, wherein Xaa22 is Lys.

[0055] As used herein, an "effective amount" is the necessary quantity to obtain positive results without causing excessively negative effects in the host to which a ligand to GHRH renal receptor may be administered. An exemplary effective amount encompassed in the present invention may relate to a quantity which may be sufficient to protect and/or treat a mammal against oxidative renal damage, prevent the death of kidney cells due to oxidative stress and/or promote regeneration of kidney cells.

[0056] An effective amount may be administered in one or more administrations, according to a regimen. The privileged method of administration and the quantity that may be administered is function of many factors. Among the factors that may influence this choice are, for example, the condition, the age and the weight of the host to which a ligand of renal GHRH receptor is to be administered. Various routes of administration may include, for example, parenteral, pulmonary, nasal, oral, transmucosal, transdermal, intramuscular, intravenous, intradermal, subcutaneous and/or intraperitonal administration.

[0057] In an embodiment of the present invention, an effective amount may not be substantially active against anterior pituitary GHRH receptor. An effective amount may have, for example, a protective effect substantially similar to a subcutaneous 1.0 mg rat GHRH(1-29)NH_2 dose per kilogram of body weight per day or lower, in a Sprague Dawley rat submitted to a high-salt diet. An effective amount may preferentially have, for example, a protective effect substantially similar to a subcutaneous 0.5 mg rat GHRH(1-29)NH_2 dose per kilogram of body weight per day or lower, in a Sprague Dawley rat submitted to a high-salt diet.

[0058] In another aspect, the present invention relates to a method for preventing (lowering) the death of kidney cells (and/or loss of kidney cell function) due to oxidative stress in a mammal in need thereof. The method may comprise administering to the mammal a ligand to GHRH renal receptor.

[0059] In a further aspect, the present invention relates to a method of promoting regeneration of kidney cells and/or function in a mammal in need thereof. The method may comprise administering a ligand to the GHRH renal receptor the mammal.

[0060] In yet a further aspect thereof, the present invention relates to the new isolated polypeptidic sequence of rat GHRH receptor, antibody that may bind same, nucleic acids that may encode same and/or vectors that may comprise the nucleic acids of the present invention.

[0061] "Polypeptides" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds (i.e., peptide isosteres). "Polypeptide" refers to both short chains, commonly referred as peptides, oligopeptides or oligomers, and to longer chains generally referred to as proteins.

[0062] The present invention therefore relates in a further aspect, to an isolated polypeptide that may comprise SEQ ID NO.: 13, SEQ ID NO.:16 or SEQ ID NO.:17, a SEQ ID NO.13, SEQ ID NO.:16 or SEQ ID NO.:17 fragment and/or a SEQ ID NO.:13, SEQ ID NO.:16 or SEQ ID NO.:17 analog. The polypeptide of the invention may be free of a N-terminal amino acid sequence of a pituitary GHRH receptor. Exemplary embodiments of the N-terminal amino acid sequence found in pituitary GHRH-R and not in renal GHRH-R are provided in SEQ ID NO.:14 and 15 for rat GHRH-R and SEQ ID NO.: 22 and SEQ ID NO.: 23 for human GHRH-R. It is to be understood that as used herein "pituitary GHRH-R" is meant to encompass both rat and/or human pituitary GHRH-R. When a polypeptide of the invention is a rat polypeptide (for example SEQ ID NO.:13) it may be free of a N-terminal amino acid sequence of a pituitary GHRH receptor from a rat. When a polypeptide of the invention is a human polypeptide (for example, SEQ ID NO.:16 or SEQ ID NO.:17) it may be free of a N-terminal amino acid sequence of a pituitary GHRH receptor from a human.

[0063] Polypeptides encompassed by the present invention may thus comprise and/or consist in SEQ ID NO.:13, SEQ ID NO.:16 or SEQ ID NO.:17, analogues of SEQ ID NO.:13, SEQ ID NO.:16 or SEQ ID NO.:17 and/or fragments thereof. Polypeptides may also comprise additional amino acids at the amino or carboxy end. These additional amino acids may be different than those of pituitary GHRH-R.

[0064] As used herein, an "analogue" is to be understood as a polypeptide which is substantially identical to an original sequence. An analogue may comprise one or more modification in the amino acid sequence in comparison with the original sequence, for example, amino acid addition(s), deletion(s), insertion(s), conservative or non-conservative substitution(s), one or more modification in the backbone or side-chain of one or more amino acid, or an addition of a group or another molecule to one or more amino acids (side-chains or backbone). An "analogue" is therefore understood herein as a molecule having a biological activity similar to that of a polypeptide described herein. Exemplary embodiments of the polypeptide analogs of SEQ ID NO.13, SEQ ID NO.:16 or SEQ ID NO.:17 may be those which possess between 60% to 100% but preferably at least 70% amino acid identity with SEQ ID NO.13, SEQ ID NO.:16 or SEQ ID NO.:17, at least 75% amino acid identity with SEQ ID NO.13, SEQ ID NO.:16 or SEQ ID NO.:17, at least 80% amino acid identity with SEQ ID NO.13, SEQ ID NO.:16 or SEQ ID NO.:17, at least 85% amino acid identity with SEQ ID NO.13, SEQ ID NO.:16 or SEQ ID NO.:17, at least 90% amino acid identity with SEQ ID NO.13, SEQ ID NO.:16 or SEQ ID NO.:17 and/or at least 95% amino acid identity with SEQ ID NO.13, SEQ ID NO.:16 or SEQ ID NO.:17. The present invention relates to and explicitly incorporates each and every specific member and combination of sub-ranges whatsoever. Thus, any specified range is to be understood as a shorthand way of referring to each and every member of a range or group individually as well as each and every possible sub-ranges encompassed therein; and similarly with respect to any sub-ranges therein. As such, with respect to amino acid identity, at least 70% encompasses any value between 70% to 100%, for example, 71%, 71.5%, 72%, 73%, 77%, 83%, etc.

[0065] An "analogue" may have sequence similarity and/or sequence identity with that of an original sequence or a portion of an original sequence. The degree of similarity between two sequences is based upon the percentage of identities (identical amino acids) and of conservative substitution. Similarity or identity may be compared, for example, over a region of 10, 20, 100 amino acids or more (and any number therebetween) or over the total length of the protein. Identity may include amino acids which are identical to the original peptide and which may occupy the same or similar position when compared to the original polypeptide. For example, a polypeptide may share 60% sequence identity with another and may have one or more conservative amino acids substitutions in the non-identical positions which may result in the polypeptide having at least 60.1%, 65%, 70%, 80%, 85%, 90% etc., sequence similarity. The remaining amino acids of the polypeptide which are neither identical nor similar may be occupied by non-conservative amino acid substitutions or alternatively by gaps (no amino acids).

[0066] Percent identity may be determined, for example, with n algorithm GAP, BESTFIT, or FASTA in the Wisconsin Genetics Software Package Release 7.0, using default gap weights. Other suitable programs for calculating the percent identity or similarity between sequences are generally known in the art, for example, another alignment program is BLAST, used with default parameters. For example, BLASTN and BLASTP can be used using the following default parameters: genetic code=standard; filter=none; strand=both; cutoff=60;

expect=10; Matrix=BLOSUM62; Descriptions=50 sequences; sort by=HIGH SCORE; Databases=non-redundant, GenBank+EMBL+DDBJ+PDB+GenBank CDS translations+Swiss protein+Spupdate+PIR. Details of these programs can be found at the following internet address: http://www.ncbi.nlm.qov/cgi-bin/BLAST.

[0067] As is generally understood, naturally occurring amino acids may be sub-classified as acidic, basic, neutral and polar, or neutral and non-polar. Furthermore, three of the encoded amino acids are aromatic. It may be of use that encoded polypeptides differing from the determined polypeptide of the present invention contain substituted codons for amino acids, which are from the same type or group as that of the amino acid to be replaced. Thus, in some cases, the basic amino acids Lys, Arg and His may be interchangeable; the acidic amino acids Asp and Glu may be interchangeable; the neutral polar amino acids Ser, Thr, Cys, Gln, and Asn may be interchangeable; the non-polar aliphatic amino acids Gly, Ala, Val, Ile, and Leu are interchangeable but because of size Gly and Ala are more closely related and Val, Ile and Leu are more closely related to each other, and the aromatic amino acids Phe, Trp and Tyr may be interchangeable.

[0068] It is known in the art that analogues may be generated by substitutional mutagenesis and retain the biological activity of the polypeptides of the present invention. These analogues have at least one amino acid residue in the protein molecule removed and a different residue inserted in its place. Examples of substitutions identified as "conservative substitutions" are shown in Table 1. If such substitutions result in a change not desired, then other type of substitutions, denominated "exemplary substitutions" in Table 1, or as further described herein in reference to amino acid classes, are introduced and the products screened.

TABLE 1

Amino Acid Substitution

Original residue	Exemplary substitution	Conservative substitution
Ala (A)	Val, Leu, Ile	Val
Arg (R)	Lys, Gln, Asn	Lys
Asn (N)	Gln, His, Lys, Arg	Gln
Asp (D)	Glu	Glu
Cys (C)	Ser	Ser
Gln (Q)	Asn	Asn
Glu (E)	Asp	Asp
Gly (G)	Pro	Pro
His (H)	Asn, Gln, Lys, Arg	Arg
Ile (I)	Leu, Val, Met, Ala, Phe, norleucine	Leu
Leu (L)	Norleucine, Ile, Val, Met, Ala, Phe	Ile
Lys (K)	Arg, Gln, Asn	Arg
Met (M)	Leu, Phe, Ile	Leu
Phe (F)	Leu, Val, Ile, Ala	Leu
Pro (P)	Gly	Gly
Ser (S)	Thr	Thr
Thr (T)	Ser	Ser
Trp (W)	Tyr	Tyr
Tyr (Y)	Trp, Phe, Thr, Ser	Phe
Val (V)	Ile, Leu, Met, Phe, Ala, norleucine	Leu

[0069] A "fragment" is to be understood herein as a polypeptide originating from a portion of an original or parent sequence or from an analogue of said parent sequence. Fragments encompass polypeptides having truncations of one or more amino acids, wherein the truncation may originate from the amino terminus (N-terminus), carboxy terminus (C-terminus), or from the interior of the protein. A fragment may comprise the same sequence as the corresponding portion of the original sequence. Fragments may be useful for example, in the generation of antibodies and/or for testing antibodies.

[0070] In another aspect, the present invention relates to an isolated nucleic acid sequence encoding a polypeptide described herein (e.g., SEQ ID NO.: 13, a SEQ ID NO:13 fragment and/or a SEQ ID NO.:13 analog, SEQ ID NO.: 16, a SEQ ID NO.16 fragment and/or a SEQ ID NO.:16 analog, SEQ ID NO.: 17, a SEQ ID NO.17 fragment and/or a SEQ ID NO.:17 analog, etc.).

[0071] In yet another aspect, the present invention relates to an isolated nucleic acid that may be selected from the group that may consist of a. a polynucleotide comprising or consisting of SEQ ID NO.11, SEQ ID NO.12, SEQ ID NO.:18, SEQ ID NO.:19, SEQ ID NO.:20 or SEQ ID NO.:21, b. a polynucleotide comprising a sequence substantially identical to SEQ ID NO.11, SEQ ID NO.12, SEQ ID NO.:18, SEQ ID NO.:19, SEQ ID NO.:20 or SEQ ID NO.:21, and/or c. a polynucleotide comprising a sequence substantially complementary to a. or b., and/or d. a fragment of any one of a., b. or c.

[0072] As used herein the term "polynucleotide" or "nucleic acid" generally refers to any polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA, or modified RNA or DNA. "Polynucleotides" or "nucleic acids" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is a mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" or "nucleic acid" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term "polynucleotide" or "nucleic acid" also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications may be made to DNA and RNA; thus "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found or not in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" or "nucleic acid" includes but is not limited to linear and end-closed molecules. "Polynucleotide" or "nucleic acid" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

[0073] In accordance with the present invention, the nucleic acid may be free of a nucleic acid sequence encoding a N-terminal amino acid sequence of a pituitary GHRH receptor as described herein.

[0074] In an additional aspect, the present invention relates to a vector that may comprise the nucleic acid sequences described herein. "Vector" refers to an autonomously replicating DNA and/or RNA molecule into which foreign DNA and/or RNA fragments are inserted and then propagated in a host cell for either expression or amplification of the foreign DNA and/or RNA molecule. The term vector may comprise, for example and without limitation, a plasmid (e.g., linearized or not) that may be used to transfer DNA sequences from one organism to another.

[0075] The term "substantially identical" used to define the polynucleotides of the present invention refers to polynucleotides which have, for example, from 50% to 100% sequence identity and any range therebetween but preferably at least 80%, at least 85%, at least 90%, at least 95% sequence identity and also include 100% identity with that of an original sequence (including sequences 100% identical over a portion of the sequence or over the entire length of the polynucleotide sequence). The present invention relates to and explicitly incorporates each and every specific member and combination of sub-ranges whatsoever. Thus, any specified range is to be understood as a shorthand way of referring to each and every member of a range or group individually as well as each and every possible sub-ranges encompassed therein; and similarly with respect to any sub-ranges therein. As such, with respect to polynucleotides sequence identity, at least 80% encompasses any value between 80% to 100%, for example, 81%, 81.5%, 82%, 83%, 87%, 93%, etc.

[0076] "Substantially identical" and "substantially complementary" polynucleotide

[0077] (nucleic acid) sequences may be identified by providing a probe of about 10 to about 20 or about 10 to about 25 nucleotides long (or longer) based on the sequence of SEQ ID NO:11 or 12 or complementary sequence thereof and hybridizing a library of polynucleotide (e.g., cDNA or else) originating from another species, tissue, cell, individual, etc. A polynucleotide which hybridizes under highly stringent conditions (e.g., 6XSCC, 65° C.) to the probe may be isolated and identified using methods known in the art.

[0078] As used herein the terms "sequence complementarity" refers to (consecutive) nucleotides of a nucleotide sequence which are complementary to a reference (original) nucleotide sequence. The complementarity may be compared over a region and/or over the total length of a nucleic acid sequence.

[0079] The term "substantially complementary" used to define the polynucleotides of the present invention refers to polynucleotides which have, for example, from 50% to 100% sequence complementarity and any range therebetween but preferably at least 80%, at least 85%, at least 90%, at least 95% sequence complementarity and also include 100% complementarity with that of an original sequence (including sequences 100% complementarity over the entire length of the polynucleotide sequence).

[0080] A further aspect of the present invention relates to an antibody capable of specific binding to polypeptides described herein. For example, an antibody capable of specific binding to SEQ ID NO.: 13, SEQ ID NO.:16 and/or SEQ ID NO.:17. Exemplary embodiments of such antibodies are those which do not substantially bind to a GHRH pituitary receptor but bind to a GHRH renal receptor.

[0081] As used herein the term "antibody" encompasses a monoclonal antibody, a polyclonal antibody, a single chain antibody, a chimeric antibody, a humanized antibody, a deimmunized antibody, an antigen-binding fragment, a Fab fragment, a $F(ab')_2$ fragment, a Fv fragment, complementarity determining regions (CDRs), or a single-chain antibody comprising an antigen-binding fragment (e.g., a single chain Fv).

[0082] The antibody may originate for example, from a mouse, rat or any other mammal or from other sources such as through recombinant DNA technologies. The antibody may also be a human antibody which may be obtained, for example, from a transgenic non-human mammal capable of expressing human Ig genes. The antibody may also be a humanised antibody which may comprise, for example, one or more complementarity determining regions of non-human origin. It may also comprise a surface residue of a human antibody and/or framework regions of a human antibody. The antibody may also be a chimeric antibody which may comprise, for example, variable domains of a non-human antibody and constant domains of a human antibody.

[0083] The minimum size of peptides useful for obtaining antigen specific antibodies may vary widely. The minimum size must be sufficient to provide an antigenic epitope that is specific to the protein and/or polypeptide. The maximum size is not critical unless it is desired to obtain antibodies to one particular epitope. For example, a large polypeptide may comprise multiple epitopes, one epitope being particularly useful and a second epitope being immunodominant, etc. Typically, antigenic peptides selected from the present proteins and polypeptides will range without limitation, from 5 to about 100 amino acids in length or may comprise the whole protein. More typically, however, such an antigenic peptide will be a maximum of about 50 amino acids in length, and preferably a maximum of about 30 amino acids. It is usually desirable to select a sequence of about 6, 8, 10, 12 or 15 amino acids, up to about 20 or 25 amino acids (and any number therebetween).

[0084] To obtain polyclonal antibodies, a selected animal may be immunized with a protein and/or polypeptide. Serum from the animal may be collected and treated according to known procedures. Polyclonal antibodies to the protein or polypeptide of interest may then be purified by affinity chromatography. Techniques for producing polyclonal antisera are well known in the art.

[0085] Monoclonal antibodies (MAbs) may be made by one of several procedures available to one of skill in the art, for example, by fusing antibody producing cells with immortalized cells and thereby making a hybridoma. The general methodology for fusion of antibody producing B cells to an immortal cell line is well within the province of one skilled in the art. Another example is the generation of MAbs from mRNA extracted from bone marrow and spleen cells of immunized animals using combinatorial antibody library technology.

[0086] In yet a further aspect, the present invention relates to an isolated cell expressing the polynucleotides and/or polypeptides of the invention. In an exemplary embodiment, the isolated cell may be a renal cell.

[0087] In another aspect, the present invention relates to an assay for identifying a ligand capable of specific binding to polypeptides of the present invention and not to pituitary GHRH receptor. This assay may comprise contacting a test ligand (e.g. a library of test ligands) with at least one polypeptide of the present invention, measuring binding of the test ligand to the polypeptide and/or determining the identity of the test ligand. The assay may further comprise a step of selecting a ligand which does not substantially bind to pituitary GHRH-R.

[0088] In a further aspect, the present invention relates to a method for the diagnosis of renal oxidative stress and/or damage. The method may comprise the step of detecting (measuring) the expression of renal GHRH receptor by any means known to a person skilled in the art, wherein downregulation of GHRH-R in early stages of suspected renal oxidative stress/damage and/or upregulation of GHRH-R in late stages of suspected renal oxidative stress/damage is indicative of renal oxidative stress and/or damage. For example, the expression of GHRH-R may be detected at the RNA (for example, mRNA) and/or protein level. The expression of mRNAs may be detected using methods which are known in the art, such as, for example and without limitation, hybridization analysis using oligonucleotide probes, reverse transcription and in vitro nucleic acid amplification methods. The expression of proteins of the invention may be detected, for example and without limitation, using antibodies specific for renal GHRH-R in a variety of methods known to a person skilled in the art of measuring polypeptides, including immunoblotting, ELISA, radioimmunassay, and FACS, etc. The present invention also encompasses a diagnosis kit that may comprise any detection reagents used to detect the expression of renal GHRH receptor (for example, a renal GHRH-R specific probe and/or a renal GHRH-R specific antibody) as well as other suitably packaged reagents and materials needed for detection of renal GHRH receptor expression.

EXAMPLES

[0089] The following examples are presented to illustrate the invention but it is not to be considered as limited thereto.

Example 1

Materials and Methods

[0090] All material and methods used in subsequent examples are herein described for reference.

[0091] Animal Handling, Treatments and Tissue Preparations

[0092] Two-month-old male Sprague Dawley rats (Charles River Canada, St-Constant, QC) were kept in temperature- (22° C.), humidity- (65%) and lighting- (12h cycles; lights on at 0700 h) controlled rooms and had free access to standard rat chow (2018 Tecklad global 18% protein rodent diet, containing 0.23% Na^+ and 0.4% Cl^-; Harlan Tecklad, Madison, Wis.) and tap water. Rats were acclimatized ≈3 days before going on a high-NaCl diet or water deprivation. Rats fed the custom-made high-NaCl diet for 2, 7 or 14 (8% NaCl; Harlan Tecklad) were compared to rats fed the custom-made control diet (0.3% NaCl; Harlan Tecklad). They had free access to water. Rats deprived of water for 3 or 5 days had free access to 2018 Tecklad rat chow. Rats used in the first series of experiments, to quantify GHRH-R mRNA levels following a 8%-NaCl diet or a water deprivation, were housed individually in metabolic cages for the entire duration of intervention. Body weight (BW), food and water intakes, and urine volume were recorded daily, and Na^+ levels were analyzed on the last 24h urine sample before sacrifice. Rats used in the 8%-NaCl diet/GHRH study were housed individually in plastic cages and BW and food intake were recorded daily. Rats were sacrificed in a block-design fashion between 0900-1130 h, by rapid decapitation. Pituitaries, kidneys and livers were excised immediately and anterior pituitaries and renal medullas dissected out. Tissues were snap-frozen in liquid nitrogen and stored at −80° C. until RNA extraction. For isolation of thin and thick limb cells, renal medullas were dissected out rapidly, washed and minced in ice-cold oxygenated HEPES-Ringer buffer (290 mosm, pH 7.4). For isolation of thick ascending limb cells, inner stripes of outer medullas were dissected out and kept in ice-cold oxygenated Hanks solution.[55] For in vivo BrdU-labeling experiments, rats were fed a 0.3%- or 8%-NaCl chow for 2 days (day 1, day 2) and received in the back a subcutaneous (sc) injection of 1.0 mg rGHRH(1-29)NH_2/kg BW, solubilized in normal physiological saline (GHRH-treated) or an isovolumetric amount of saline (control). rGHRH-(1-29)NH_2 (synthesized in our laboratory)[56] was solubilized each morning just before treatment and kept on ice. Rats were injected intra-peritoneally on the morning of day 3, with 100 mg 5-bromo2'-deoxy-uridine/kg BW (30 mg ultrapure BrdU/1 ml in normal saline; Sigma-Aldrich Canada Ltd, Oakville, ON), 2 h prior to sacrifice. For in vivo GHRH treatment, rats received in the back a subcutaneous (sc) injection of 1.0 mg rGHRH(1-29)NH_2/kg BW daily.

[0093] Porcine anterior pituitaries and renal medullas from Yorkshire-Landrace pigs (≈107 kg, ≈150-day-old) were dissected out at a local slaughter house, snap-frozen in liquid nitrogen and stored at −80° C. until RNA extraction.

[0094] Isolation of Thin Limbs of Henle's Loop Cells

[0095] Cell dispersion of minced medullas to obtain semi-purified thin limb cells was performed as previously described.[33] These cells were used immediately for in vitro determination of basal and rGHRH(1-29)NH_2-induced cAMP levels or purified by differential centrifugation for immunocytochemistry, using a continuous gradient of Nycodenz. The gradient was prepared as described by Grupp et al.[55] and thin limb cells were recovered in fraction I of the gradient after centrifugation at 1500 g (16° C., 45 min) and washed twice in HEPES-Ringer buffer (430 g, 16° C., 10 min). Cell viability, assessed by the Trypan Blue exclusion method, was around 95%. When purified thin limb cells were cultured, isolation and purification steps were performed under sterile conditions and media containing antibiotics.

[0096] Isolation of Thick Limbs of Henle's Loop Cells

[0097] The inner stripe of outer medullas were dissected out using an optical stereomicroscope, minced and kept in oxygenated Hanks solution. Short time cell dispersion was performed as previously described,[57] and dispersed cells were poured on the top of a 100 μm-pore nylon membrane (Millipore, Nepean, ON, CA) and washed with Hanks-1% BSA (Sigma-Aldrich) solution using a syringe adapted to a 25G needle. Thick ascending HL cells were detached from the membrane by washing with Hanks-1% BSA solution. The suspension was centrifuged at 80 g for 5 min (4° C.) and the pellet resuspended in ice-cold Hanks solution. Cell viability was determined as above and was similar.

[0098] Immunocytochemical Procedures

[0099] Specific markers of descending (anti-aquaporin-1 antiboby (Ab)) and ascending thin limb (anti-CIK-K1/-K2 (CIC-K) Ab) cells (Alamone Labs, Jerusalem, Israel) were directly conjugated to the fluorochrome Alexa 488, using the Alexa™ 488 Protein Labeling Kit (Molecular Probes, Eugene, Oreg.), according to the manufacturer's protocol. Labeled antibodies were purified on molecular size exclusion spin columns, supplied with the kit (1100 g, 5 min). Purified thin limb cells were fixed in fresh 4% paraformaldehyde-phosphate-buffered saline (20 min, RT), washed twice with PBS and centrifuged (800 g, 4° C., 5 min). Thin limb cells (≈2200,000) were spun onto glass slides by cytocentrifugation (32 g, RT, 2 min) and permeabilized in 0.2% Triton X-100 (Sigma-Aldrich) for 15 min (RT). Slides were washed in PBS (4×5 min, RT), blocked with 5% (wt/vol.) BSA-PBS (30 min, RT) and washed in PBS (2×10 min). GHRH-R was detected using 0.5 μg of the purified anti-GHRH-R(392-404) polyclonal antibody[33] in 100 μl PBS, containing 1% BSA, incubated overnight (ON) at 4° C., in a humid atmosphere (7, 8). Cells were rinsed in PBS (2×10 min, RT), incubated 60 min (RT), in the presence of Alexa 568™-conjugated goat anti-rabbit IgGs (Molecular Probes) (1:15000 in PBS-BSA 1% buffer) and washed in PBS (2×10 min). Descending thin limb cells were then visualized using a rabbit polyclonal Alexa 488™-conjugated anti-aquaporin-1 antibody (1:2000 diluted in PBS-BSA 1%, 60 min, 37° C.) while ascending thin limb cells were visualized using a rabbit polyclonal Alexa 488™-conjugated anti-ClC-K antibody (1:500 diluted in PBS-BSA 1%, 60 min, 37° C.). A final wash of slide-mounted cells was done in PBS (2×10 min). Specificity of labeling was assessed by substituting GHRH-R (392-404) polyclonal antibodies with normal IgGs. Another series of experiments, using a similar procedure as described above, was performed to determine whether or not immunoreactive GHRH was present in ascending thin limb cells. An anti-rat GHRH(1-43) OH antibody (0.5 μg/100 μl; Bachem Biosciences Inc, King of Prussia, Pa.) and a secondary Alexa 568-conjugated goat-anti-rabbit IgGs (1/7500, Molecular Probes) were used. One μM of 4,6-diamodino-2-phenylindole dihydrochloride (DAPI, Molecular Probes) was added for the last 30 min of incubation to stain nuclei. All procedures with fluorescent probes were performed in the dark. Cells were visualized using a Nikon Eclipse E600 (Nikon Canada Inc., Montreal, QC) fluorescence/light microscope equipped with filters for excitation/emission of fluorescein (485/520 nm) and Texas Red (595/660) and DAPI (360/460 nm).

[0100] BrdU immunolabeling (Roche Diagnostics, Laval, QC, CA) was used to quantify DNA repair/synthesis in purified thin limb cells from rat submitted 2 days to a 8%- or 0.3%-NaCl diet and injected with GHRH or saline. The cells were purified as above, cultured 16h in DMEM/F12, containing 25 mM glucose, 10% fetal bovine serum, 1% penicillin-streptomycin, 0.1% amphotericin,[33] on coverslip in 24-wells sterile culture plates ($\approx 1\times10^6$ cells/well). They were fixed with fresh 4% paraformaldehyde (500 μl/well, 15 min, RT) and washed using the washing buffer supplied with the kit (2×5 min, 500 μl/well). They were subsequently incubated in blocking buffer as above (30 min, RT, 500 μl/well). Immunolabelling was performed with the primary antibody anti-BrdU (dilution 1:10 in the incubation buffer, 30 min, 37° C., 150 μl/well). Non-specific fluorescence was determined by substituting the primary antibody by normal rabbit IgGs. Immunodetection was performed after washing by adding a secondary anti-rabbit-IgG antibody coupled to fluorescein (30 min, 37° C., 150 μl/well). All steps using fluorescent labeling was performed in the dark. Nuclei and mitochondria were labeled using 1 μM DAPI and 10 nM of Mitotracker red CMXRos (Molecular Probes, Oreg., USA; PBS 1×, 15 min, RT, 200 μl/well). After final washing, cells on coverslips were dried and mounted with Prolong mounting medium/Prolong antifade (Molecular Probes). Slide-mounted cells were kept 16-24h at room temperature and stored at 4° C. in the dark. Cells were visualized and fluorescence intensity was quantified using fluorescence microscopy as described above. Intensity of fluorescence and occurrence of co-labeling were analyzed using the Metamorph 4.5 software (Universal Imaging Corporation, Canberra Packard Canada LTD, Mississauga, ON, CA. A 6-level (0 to 5) intensity scale was used to assess fluorescence intensities: background (level 0): 0-43 pixels, very weak (level 1); 44-85 pixels, weak (level 2); 86-128 pixels, moderate (level 3); 129-170 pixels, high (level 4); 171-213 pixels and very high (level 5): 214-255 pixels), as previously described.[58] Total fluorescence was determined for each image using arbitrary density units defined as: Σ (% cell labeled X intensity level). Levels 3-5 were considered as immunospecific.

[0101] Ribonuclease Protection Assay of Renal GHRH-R

[0102] Total RNA from medullas and purified descending and ascending thin limb cells was extracted with TRIzol (Invitrogen Canada, Burlington, ON). GHRH-R mRNA levels were assessed using the RPR64 probe corresponding to the 3'-end of the rat GHRH-R complementary DNA (cDNA) (nucleotide position: 1044-1611; Genbank accession number: L01407).[2] The ribonuclease protection assay was performed as previously described.[33] Twenty μg total RNA from renal medulla or purified thick HL or liver, 5 μg total RNA from purified thin limb cells or anterior pituitary were used. Tissue GHRH-R and GAPDH mRNA and cRNA external standard levels were quantified by densitometry. GHRH-R mRNA levels were always normalized with both GAPDH mRNA internal and GHRH-R cRNA external standards, in order to maintain an intra-assay coefficient of variation ≦10% in all experiments. Specificity of the $[^{32}P]$GHRH-R probe was assessed in each experiment using positive (5 μg pituitary total RNA) and negative (20 μg liver total RNA) controls. In addition, linearity of protected signals was assessed in each experiment, using 10-30 μg medulla total RNA. Results were expressed in percentage of relative density to the control condition or tissue preparation, using a fixed amount of total RNA, which reflects the concentration of GHRH-R mRNA at cellular level. Since changes may either be compensated or aggravated at the organ/tissue level, results were also expressed as total GHRH-R mRNA relative densities per renal medulla total RNA content, to document physiological impacts of interventions.[33]

[0103] Northern Blot Hybridization of Anterior Pituitary GHRH-R

[0104] Total RNA was extracted as above. Northern blot hybridization was performed as previously described on 12 μg total RNA samples with minor modifications.[33] Prehybridization was performed in Robbins' hybridization solution (7% SDS containing 0.25 M Na_2HPO_4 (pH 7.4), 1 mM EDTA (pH 8.0) and 1% BSA) at 65° C., 2 h. Hybridization was performed in fresh Robbins' solution at 65° C. (ON), in the presence of labeled RPR64. Membranes were subsequently washed, exposed to films, stripped and rehybridized with GAPDH 28S probes.[33] Quantification of each GHRH-R mRNA transcript (2.5 and 4 kb), GAPDH mRNA and 28S rRNA levels was performed by densitometry. GHRH-R mRNA levels were normalized with 28S rRNA in all experiments, to maintain the intra-assay coefficient of variation ≦10%. Specificity of the $[^{32}P]$RPR64 cDNA probe was assessed in each experiment using 5 μg liver total RNA. Linearity of protected signals was measured routinely, using 6-18 μg total RNA. Results were expressed in percentage of relative density to that of control groups, using a fixed amount of total RNA. Results were also expressed as total GHRH-R mRNA relative densities per anterior pituitary total RNA content.

[0105] Reverse Transcriptase-PCR of preproGHRH

[0106] Total RNA from purified thin limb cells (2 μg) was subjected to two steps RT-PCR using SuperScript™ First-Strand Synthesis System (Invitrogen). Reverse transcription was performed using SuperScript™ II RT and PCR reaction was performed using Platinum® Taq DNA polymerase according to manufacturer's protocol (First-Strand synthesis using oligo(dT) PCR for targets up to 4 kb). PCR reaction was performed on a 1:5 dilution of the first strand cDNA product in a final volume of 50 µl containing 0.4 µl of Platinum® Taq DNA polymerase. Reagents were added to a final concentration of 1×PCR buffer [20 mM Tris-HCl (pH 8.4), 50 mM KCl], 1.5 mM $MgCl_2$, 0.2 mM dNTPs and 0.3 µM sense and antisense desalted primers diluted in sterile picopure water (GAPDH sense 5'-gggtgtgaaccacgagaaat-3', GAPDH antisense 5'-actgtggtcatgagcccttc-3', nt 1242-1376 GenBank NM_017008; preproGHRH sense 5'-atgccactctgggtgttcttt-3', preproGHRH antisense 5'-gcagtttgcgggcatataat-3', nt 196-352 GenBank NM_031577). The reaction was performed in Biometra TGradient PCR (Montreal Biotech Inc, Montreal, QC) with the following cycle profile: denaturation at 94° C. for 2 min, followed by 39 cycles of denaturation at 94° C. for 30 sec, annealing at 58° C. for 70 sec, extension at 72° C. for 60 sec and a final cycle at 94° C. for 30 sec, 58° C., 60° C., and 62° C. for 60 sec and a 5-min extension at 72° C. PreproGHRH and GAPDH PCR products were analyzed by gel electrophoresis on 2% agarose gel containing 0.5 µg/ml of ethidium bromide with a 100 bp molecular weight standard (Invitrogen).

[0107] RT-PCR of GHRH-R

[0108] Total RNA (2 µg) from purified aTL cells was subjected to two steps RT-PCR using SuperScript™ First-Strand Synthesis System for RT-PCR (Invitrogen/Canada Life Technologies). Reverse transcription was performed using SuperScript™ II RT and PCR reaction was performed using Platinum® Taq DNA polymerase according to manufacturer's protocol (First-Strand synthesis using oligo(dT) or GSP and PCR for targets up to 4 Kb). PCR reaction was performed on a 1:5 dilution of the first strand cDNA product in a final volume of 50 uL containing 0.4 ul of Platinum® Taq DNA polymerase. Reagents were added to a final concentration of 1×PCR buffer (20 mM Tris-HCl (pH 8.4), 50 mM KCl], 1.5 mM $MgCl_2$, 0.2 mM dNTPs) and 0.3 uM sense and antisense desalted primers from the rat (three sets of primers covering the 5', middle portion and 3' regions were used (PubMed NM_012850): nt 58-191 (5'-ctctgcttgctgaacctgtg-3' (sense (s)), 5'-catcccatggacgagttgtt-3' (antisense (as)); nt 578-736 (5'-ctgctgtcttccagggtgat-3' (s), 5'-taggagatgtggaggccaac-3' (as)); nt 1064-1227 (5'-acttcctgcctgacagtgct-3' (s), 5'-tggcagaagttcagggtcat-3' (as)), and porcine pituitary GHRH-R (three sets of primers three sets of rat primers covering the 5', middle portion and 3' regions were used (PubMed L11869: nt 144-271 (5'-ctgctgagctccctaccagt-3' (s), 5'-cagcccgaggaggagttg-3' (as)); nt 694-816 (5'-gcttctccacggttctgtgca-3' (s), 5'-tgggtgacgtagaggccaag-3'(as)); nt 1201-1342 (5'-gctccttccagggcttcattgt-3' (s), 5'-gaaggctttgcccatttggca-3' (as)) cDNA sequence were diluted in sterile picopure water. The reaction was performed in Biometra TGradient PCR (Montreal Biotech Inc) with the following cycle profile: denaturation at 94° C. for 2 min, followed by 39 cycles of denaturation at 94.0 for 30 sec, annealing at 58.0° C., 60.0° C., and 62.0° C. for 70 sec, extension at 72° C. for 60 sec and a final cycle at 94° C. for 30 sec, 58.0° C., 60.0° C., and 62.0° C. for 60 sec and a 5-min extension at 72° C. GHRH-R, GAPDH and GHRH PCR products were analyzed by gel electrophoresis on 2% agarose gel containing 0.5 ug/ml of ethidium bromide with a 100 bp molecular weight standard (Invitrogen Life Technologies, Burlington, ONT, CA). The gel was visualized using a IS1000 Digital imaging system (Alpha Innotech Corp./Canberra Packard).

[0109] Quantitative Real-Time RT-PCR of GHRH-R and ClCK-1

[0110] Total RNA from purified thin limb cells was extracted with TRIzol. Samples were resuspended in RNAse-free water (Ambion). Reverse transcription of 2 µg total RNA was performed using the SuperScript™ II RT kit (Invitrogen) and random hexamer primers, according to the manufacturer's protocol, including RNAse H treatement. mRNA levels of rat GHRH-R and rat GAPDH (internal control) were determined in separate tubes, by real-time PCR, using a 1/150 (GHRH-R) and 1/300 (GAPDH) dilution of the RT product and the reagents from the Quantitect™ SYBR® Green PCR kit (Qiagen, Mississauga, ON, CA), according to the manufacturer's recommendation. The ABI protocol was used except that the dUTP/uracil-N-glycosylase step was omitted. Reactions were performed in duplicate, in a final volume of 25 µL, containing 300 nM of sense and antisense primers, using a Rotor Gene 3000 real-time thermal cycler (Montreal Biotech Inc, Montreal, QC, CA). No template and no amplification controls were always included to confirm the specificity of reactions. The parameters included a single cycle of 95° C. for 15 min, followed by 45 cycles of 94° C. for 15 sec, annealing at 52° C. for 30 sec, extension at 72° C. for 30 sec and a melting step going from 72° C. to 99° C. (ramping at 1° C./sec). Specific primers (300 nM) for GHRH-R, ClCk-1 and GAPDH were used. Specificity of the PCR products was established by melting curve analysis and by running products on 2% agarose gel, containing 0.5 µg/ml of ethidium bromide, with a 100 bp molecular weight standard (Invitrogen). Results were analyzed using the Rotor-Gene application software (version 6.0). A five-point standard curve was performed for each gene tested, using 1:5 serial dilutions (1:5 to 1:3125) of renal medulla total RNA from 2-month-old healthy male rats. The intra-assay coefficient of variation of GHRH-R and GAPDH Ct values was $\leqq$2.5% in all experiments

[0111] Quantification of cAMP Levels in Semi-Purified Thin Limbs of Henle's Loop Cells

[0112] Sensitivity to GHRH was assessed in freshly semi-purified thin limb cells[33] from rats fed a 8%-NaCl diet for 2, 7 or 14 days or the control diet. Cells (1×10^6 cells=60-75 µg prot/ml/Eppendorf tube) were preincubated 30 min (37° C.) in 1 ml DMEM/F12 cultured media,[33] containing 1× antibiotics, 0.2% BSA and 1 mM isobutyl-1-methylxanthine (IBMX, Sigma) and challenged 15 min (37° C.) with 1 and 100 nM GHRH, the vehicule (DMEM/F12-0.2% BSA) or 10 µM forskolin to assess the reactivity of cell preparation. The reaction was stopped by centrifugation (5 min, 4° C., 12 000 g). Pellets were resuspended in 200 µl of lysis buffer (10 min, RT, vortex) supplied with the EIA kit (cAMP Direct Biotrak™ enzyme immunoassay kit, Amersham Biosciences) and centrifuged (5 min, 4° C., 12 000 g). Supernatants were used to quantify immunoreactive cAMP levels (non-acetylated method). Pellets were kept frozen for determination of protein content.[59] Optical densities were measured at 450 nm, using a microplate reader (Bio-Rad, model 3550). The intra-assay coefficient of variation was $\leqq$12% in all experiments. Net GHRH-induced cAMP levels (without basal level) were expressed in percentage of relative levels compared to that obtained in the presence of GHRH.

[0113] Quantification of Cell Proliferation in Semi-Purified Thin Limbs of Henle's Loop Cells

[0114] Freshly semi-purified cells were cultured in DMEM/F-12 culture media, containing antibiotics and the vehicle (culture medium) or 1, 10 or 100 nM rGHRH (1-29) NH_2. GHRH was added at time 0 and after a 24 and 48 h culture period. Proliferation was assessed with aliquots of 40,000 cells, after a 60-h cell culture period, using the Promega kit (CellTiter 96R Aqueous one solution cell proliferation assay).

[0115] Data Analysis and Statistics

[0116] RPA represents a more sensitive and reliable method to perform a valid quantification of GHRH-R mRNA levels in rat renal medulla and Henle's loop cells compared to Northern blotting.[33] However, Northern blotting was chosen to study the pituitary GHRH-R mRNA levels as it allows the detection of GHRH-R individual transcripts[19]. The validity of comparing GHRH-R mRNA levels, using RPA and Northern blotting was assessed using pituitary total RNA. In the pituitary from 3-day water-deprived rats, GHRH-R mRNA levels obtained from Northern blots (sum of densities of the two transcripts: 3.0±0.2 times higher than controls) were not significantly different from those obtained by RPA (sum of densities of the two protected fragments: 3.1±0.3 times higher than controls), indicating that medullary and pituitary GHRH-R mRNA levels can be compared. Quantification of GHRH-R mRNA transcripts, protected fragments and visualization of gels were performed using an IS1000 Digital imaging system (Alpha Innotech Corp/Canberra Packard, QC).

[0117] Results were expressed as mean±SEM. Comparisons of normalized GHRH-R mRNA levels as well as intracellular cAMP levels, immunofluorescence intensity, anti-BrdU immunoreactive cells were performed by ANOVA, followed by the Dunnett's multiple range test or by the unpaired Student's t test. Statistical significance of differences was established at $P<0.05$.

[0118] Rapid Amplification of GHRH-R cDNA Ends

[0119] The 5'- and 3'-Rapid Amplification of cDNA Ends (RACE)-Ready cDNAs were synthesized using the SMART RACE cDNA Amplification Kit, according to the manufacturer's recommendations (Clontech Laboratories Inc, Mountain View, Calif.). Briefly, the synthesis of cDNAs for 5' RACE was performed in a final volume of 10 µl containing 1 µg of poly$(A)^+$RNA provided from anterior pituitary, renal medulla or liver, 1.2 µM 5'-CDS (5'-$(T)_{25}$VN-3', where N=A, C, G, or T and V=A, G, or C), 1.2 µM BD SMART II A oligonucleotide (5'-AAG CAG TGG TAT CAA CGC AGA GTA CGC GGG-3'), 1× first-strand buffer, 2 mM DTT, 1 mM each dNTP, and 1 µl BD Power-script reverse transcriptase. The synthesis of 3'-RACE cDNA was performed using the same strategy using 1.2 µM 3'-CDS Primer A (5'-AAG CAG TGG TAT CAA CGC AGA GTA $C(T)_{30}$VN-3'). The 5'- and 3'-RACE ready cDNAs were diluted 1:25 in 10 mM Tricine-KOH, pH 8.5, containing 1 mM EDTA and used in subsequent amplifications.

[0120] The primary PCR included 1 µl of 5'- or 3'-RACE-Ready cDNA (1:25), 1× universal primer mix (UPM) (long: 5'-CTA ATA CGA CTC ACT ATA GGG CAA GCA GTG GTA TCA ACG CAG AGT-3'; short: 5'-CTA ATA CGA CTC ACT ATA GGG C-3') that recognized the SMART II oligonucleotide sequence, inserted in both 5' and 3' during cDNA synthesis, 0.2 µM rat GHRH-R gene-specific primer (Table 2: Ex-9/10P for 5'-RACE-Ready cDNA and Ex-7bP for 3'-RACE-Ready cDNA), 1× Advantage 2 PCR buffer, 0.2 mM each of dNTPs and 1× BD Advantage 2 Polymerase Mix containing AdvanTaq DNA polymerase, a proofreading polymerase, and TaqStart Antibody to provide automatic hot-start PCR (Clontech Laboratories Inc) in a total volume of 50 µl. All GHRH-R gene-specific primers used in PCR experiments (Table 2) were synthesized by Invitrogen Canada Inc. To improve the specificity of RACE, a touchdown PCR (Biometra instrument) was performed with the following cycle profile: 5 cycles of 94.0° C. (denaturation) for 30 sec and 72.0° C. (annealing and extension) for 3 min, 5 cycles of 94.0° C. for 30 sec, 70.0° C. (annealing) for 30 sec, and 72.0° C. (extension) for 3 min, followed by 20 cycles of 94.0° C. for 30 sec, 68.0° C. (annealing) for 30 sec, and 72.0° C. (extension) for 3 min. The primary PCR product was diluted 1:50 with Tricine/EDTA buffer. To further increase the specificity and sensitivity of amplification, secondary PCR was carried out with 5 µl or primary PCR product, 0.2 µM nested universal primer (NUP) (5'-AAG CAG TGG TAT CAA CGC AGA GT-3'), and 0.2 µM nested gene-specific primer (N) (Table 2: Ex-9P for 5'-RACE product and a primer complementary to Ex-7/8P for 390'-RACE product) in a total volume of 50 µl. The nested-PCR was performed using 20 cycles of denaturation at 94.0° C. for 30 sec, annealing at 68.0° C. for 30 sec and extension at 72.0° C. for 3 min.

TABLE 2

Sequence of GHRH-R gene-specific primers

ID	Application	Sequence (5'-3')	Position on pituitary GHRH-R cDNA	Position on chromosome 4 (AC091710)	Direction
Ex-1P	Characterization	Ctctgcttgctgaacctgtg SEQ ID NO.:	58-77	38112-38131	S
Ex-2/3P	Characterization	Catcccatggacgagttgtt SEQ ID NO.:	172-191	40807-40822/40933-40936*	A S
Ex-6P	Characterization/validation	Ctgctgtcttccagggtgat SEQ ID NO.:	578-597	43668-43687	S

TABLE 2-continued

Sequence of GHRH-R gene-specific primers

ID	Application	Sequence (5'-3')	Position on pituitary GHRH-R cDNA	Position on chromosome 4 (AC091710)	Direction
Ex-7aP	Characterization	Taggagatgtggaggccaac SEQ ID NO.:	717-736	45132-45151	AS
Ex-11P	Characterization/sequencing	Acttcctgcctgacagtgct SEQ ID NO.:	1064-1083	47288-47307	S
Ex-13P	Characterization	Tggcagaagttcagggtcat SEQ ID NO.:	1208-1227	50611-50630	AS
Ex-9/10P	5'RACE	Cagcccaaagttcaccccaacagagagg SEQ ID NO.:	924-897	46016-46028/46594-46608*	AS
Ex-9P	5'RACE (nested)	Ccagtagggggagctgtcgtctaggtc SEQ ID NO.:	873-847	45992-45966	AS
Ex-7bP	3'RACE	Ctggtggctggttctcgctggctgg SEQ ID NO.:	753-777	45168-45192	S
Ex-7/8P	3'RACE (nested)	Tctcgctggctggggactccctgtg SEQ ID NO.:	765-789	45180-45193/45480-45490	S
Ex-8P	Sequencing	Cctgtgctatgcactggta SEQ ID NO.:	824-842	45485-45503	S
Ex-1M	Validation	Agacacgggaccagaaatc SEQ ID NO.:	—	18647-18665	S
Ex-2M	Validation	Agcctttatccatgcgacag SEQ ID NO.:	—	19331-19350	S

TABLE 2-continued

Sequence of GHRH-R gene-specific primers

ID	Application	Sequence (5'-3')	Position on pituitary GHRH-R cDNA	Position on chromosome 4 (AC091710)	Direction
Ex-11M	Validation	Gatgcaaattatattgagaaacag SEQ ID NO.:	945-922	46606-46629	AS

Primers were identified according to their exon localization on the rat anterior pituitary (P) (Genebank NM_012850) or renal medulla (M) GHRH-R cDNA.

*Primer sequence localized on two adjacent exons and separated on chromosome 4 by an intronic sequence.

S: sense. AS: antisense.

[0121] Purification and Sequencing

[0122] The renal medulla and anterior pituitary 5'-RACE products were analyzed by electrophoresis on a 1% agarose gel, using TAE buffer, containing 0.5 µg/ml ethidium bromide and visualized. Each fragment was eluted using the MinElute Gel Extraction Kit (Qiagen, Mississauga, ON, CA). Purified products were inserted into the pCR4-TOPO vector and amplified in TOP10 *E. Coli* (Topo TA cloning kit, Invitrogen Inc). Amplified plasmids were purified using the Quick Lyse MiniPrep kit (Qiagen). The insert in the plasmid was sequenced using the primer pair supplied with the cloning kit (M13 forward: 5'-gtaaaacgacggccag-3'; M13 reverse: 5'-caggaaacagctatgac-3'). The renal medulla and anterior pituitary 3'-RACE products, pooled from three PCR reactions, were purified on 1% agarose gel containing 0.5 µg/ml ethidium bromide, visualized and extracted using the MinElute Gel Extraction Kit (Qiagen). The purity of each PCR product was ascertained by gel electroproresis as above, and PCR products were sequenced using specific GHRH-R primers (Table 2: Ex-8P and Ex-11P). Sequencing was performed at the CHUM Research Center core facilities, using AmpliTaq DNA polymerase FS and a 16-capillary ABI Prism 3100 fluorescent sequencer (Applied Biosystems Canada/Ambion).

[0123] General Characteristics of Rats Submitted to a High-NaCl Diet or Water Deprivation

[0124] Body weight (BW), food and water intakes, urine flow rate and urine sodium rate of rats fed a 8%-NaCl diet (GHRH-R mRNA study), and BW and food intake of water-deprived rats are reported in Table 3. As previously observed,[35,36] water intake, urine flow rate and urine sodium excretion rate of rats submitted to the 8%-NaCl diet were significantly increased when compared to controls (P<0.001). After 14-day of the high-salt regimen, BW was decreased by 7% (P<0.01). Food intake was not modified by this diet. No change was observed in BW and food intake of 2-month-old rats submitted to a 2-day 8%-NaCl diet and either injected with GHRH or saline. BW of water-deprived rats was decreased when compared to controls (P<0.001), as reported before.[37] Moreover, their food intake decreased (P<0.001), providing an explanation for the loss of BW.

TABLE 3

Characteristics of rats following a 2-, 7-, or 14-day high-NaCl diet or a 3- or 5-day water deprivation

	Control	High-NaCl	Control	High-NaCl	Control	High-NaCl	Control	Water deprivation	Control	Water deprivation
Duration (day)	2	2	7	7	14	14	3	3	5	5
Number of animals	8	7	8	8	8	8	8	9	8	9
Body weight (g)	264 ± 5	266 ± 3	323 ± 11	315 ± 14	382 ± 5	354 ± 7^{a}	242 ± 2	172 ± 2^{b}	343 ± 8	268 ± 6^{b}
Food intake (g/day)	25 ± 2	23 ± 1	25 ± 1	26 ± 1	25 ± 1	27 ± 2	22 ± 2	8 ± 2^{b}	23 ± 1	5 ± 1^{b}
Water intake (ml/day)	26 ± 2	89 ± 6^{b}	32 ± 4	109 ± 7^{b}	41 ± 2	116 ± 7^{b}	34 ± 2	0	29 ± 3	0
Urine flow rate (ml/day/100 g BW)	5.5 ± 0.7	29.8 ± 2.5^{b}	4.0 ± 0.9	26.5 ± 1.6^{b}	5.4 ± 0.8	25.9 ± 1.8^{b}	ND	ND	ND	ND
Urine$_{Na}$ rate (mmol/day/l)	ND	ND	57.6 ± 7.0	>300^{b}	ND	ND	ND	ND	ND	ND

Values are means ± SEM of the data compiled at the end of the study.

High-NaCl: 8%-NaCl chow.

Control: 0.3%-NaCl chow.

ND: not determined.

Values represent the mean ± SEM.

^{a}P < 0.01 and

^{b}P < 0.001, compared to respective controls, by two-tailed unpaired Student's t test.

Example 2

GHRH-R Expression Profile

[0125] To increase knowledge on the medullary GHRH-R and identify its cell-specific localization, purified thin and thick limbs of Henle's loop cells were prepared. GHRH-R mRNA levels were analyzed by ribonuclease protection assay (RPA) as described in Example 1.[33] Two distinct bands were detected, using the RPR64 rGHRH-R probe and their sum was considered as the total level of GHRH-R mRNA, as in previous works.[33, 38] In kidneys from 2-month-old healthy male rats (FIG. **1**), thin limb cells contained highest levels of GHRH-R mRNA. A high level of GHRH-R mRNA was detected in thin limbs only. Those found in ascending thick limb (ATL) cells and total medulla were 5.8 and 3.4 times lower, respectively ($P<0.01$). GHRH-R mRNA levels from ATL cells were 1.7 times lower than those in total medulla ($P<0.05$).

[0126] Immunocytochemical Localization of GHRH-R in Thin Limbs of Henle's Loop

[0127] As the highest level of GHRH-R mRNA was observed in thin limbs of Henle's loop (HL) cells, a purified cell preparation was used to assess the precise localization of GHRH-R. Since thin limb cells contains a descending segment participating to water transport, and an ascending segment actively involved in ion transport, it was important to identify the specific cell type expressing GHRH-R in this part of the nephron, to help defining potential roles. Co-immunolocalization of GHRH-R, with specific markers of descending (aquaporin-1)[41] and ascending (CIC-K1)[36, 42] thin limb cells was performed. Co-immunolocalization of GHRH-R with markers of the thin descending (aquaporin-1) and thin ascending (CIC-K) limbs of HL cells revealed as shown in FIG. **2** that aquaporin-1 positive cells (FIG. **2***a*) were devoid of GHRH-R (FIGS. **2***b*, **2***c*). However, CIC-K (FIG. **2***d*) and GHRH-R co-labeling was observed in of the cells (FIGS. **2***e*, **2***f*). No signal was seen when the GHRH-R or CIC-K antibody was substituted by normal IgGs (data not shown).

[0128] Immunocytochemical Localization and Gene Expression of GHRH in Thin Limbs of Henle's Loop

[0129] GHRH and CIC-K immunofluorescence was always co-localized as shown in FIG. **3**. No signal was observed when the GHRH primary antibody was substituted by normal IgGs (FIG. **3**A panel e). The GHRH fluorescent signal overlapped ≈45% of CIC-K immunoreactive cells (FIG. **3**A panel f). In addition, positive results from RT-PCR strongly suggest that immunoreactive preproGHRH is locally synthesized in thin limbs of HL (FIG. **3**B). In conclusion, GHRH-R immunofluorescence was highly co-localized with that of CIC-K but not aquaporin-1, indicating a specific expression in ascending thin limb cells. Moreover, these results show, for the first time, the presence of a local GHRH-GHRH-R system in these cells.

Example 3

In Vivo Regulation of Renal Medulla GHRH-R mRNA Levels Following a 2-, 7- or 14-Day High-NaCl Diet or a 3- or 5-Day Water Deprivation

[0130] To gain more information on potential roles of the renal GHRH-R, the regulation of medullary GHRH-R mRNA levels was studied using in vivo models of renal dysfunction by Na^+/C^- or water homeostasis disruption, as obtained with a high-NaCl diet for 2, 7 or 14 days or a water deprivation for 3 or 5 days.

[0131] At the cellular level (GHRH-R mRNA level per fixed amount of total RNA), GHRH-R mRNA concentrations were differentially regulated according to the duration of the high-salt diet. They decreased at 2 days, increased at 7 days, and returned to normal at 14 days. This is shown in FIG. **4**, wherein after a 2- and 7-day 8%-NaCl dietary intake, renal medulla GHRH-R mRNA levels were 1.4-fold lower ($P<0.01$) and 1.3-fold higher ($P<0.05$) than those of control rats (0.3% NaCl), respectively, when expressed per 20 μg total RNA, to reflect cellular levels.

[0132] The same type of change was seen when data were analyzed at the tissue level (GHRH-R mRNA levels per total medulla RNA content), indicating that the cellular effect was not counterbalanced systemically. Indeed, they were decreased by 1.5-fold after 2 days ($P<0.05$) and increased by 1.7-fold after 7 days ($P<0.01$) of the 8%-NaCl diet, when expressed per medulla total RNA content, to reflect tissue level (data not shown).

[0133] After 14 days of the regimen, no significant difference was observed between GHRH-R mRNA levels from rats submitted to the high-salt diet and controls, either expressed per 20 μg total RNA (FIG. **4** B) or per medulla total RNA content (data not shown). The effects of the high-NaCl diet were not mimicked by a 3- or 5-day water deprivation, two situations provoking hypertonicity. After 3- or a 5-day water deprivation, no significant difference was observed between GHRH-R mRNA levels from water-deprived and control rats, having free access to water (data not shown). Therefore, this indicates that the ascending thin limb GHRH/GHRH-R system is not directly involved in the regulation of ion transport.

[0134] High-salt diet regulates genes involved in higher fibrotic activity, cellular stress and apoptotis in the rat renal medulla.[46] and administration of substances exhibiting anti-oxidant properties attenuates or prevents these deleterious effects.[47, 48] Changes in GHRH-R mRNA levels and GHRH sensitivity, between 2 and 7 days of a high-NaCl diet, suggests that GHRH-R activation may promote ascending thin limb cell survival early on in a situation of oxidative stress and subsequently proliferation. The GHRH-R could, rather than regulate ion transport, participate to adaptive processes in ascending thin limb cells to compensate for an increased oxidative stress and cell damage caused by a drastic and sustained high-NaCl intake.[45]

Example 4

In Vivo Regulation of Anterior Pituitary GHRH-R mRNA Levels Following a 2-, 7- or 14-day high-NaCl Diet or a 3- or 5-Day Water Deprivation

[0135] The presence of 2.5- and 4-kb GHRH-R mRNA transcripts was observed in the anterior pituitary of all rats (controls, 8%-NaCl-fed, water-deprived), as previously reported.[10] In the pituitary from high-salt-fed rats, no drastic changes of GHRH-R mRNA transcript levels were observed when expressed per 12 μg total RNA (FIG. **5**A-C). After 7 days of the regimen, the levels of the 2.5-kb GHRH-R mRNA transcript was transiently decreased by 1.2-fold ($P<0.05$; FIG. **5**B). No change in pituitary GHRH-R mRNA levels was observed, at any time, when data were expressed per pituitary total RNA content (data not shown).

[0136] After 3 days of water deprivation, pituitary levels of the 2.5-kb GHRH-R mRNA transcript and combined levels of 2.5-kb and 4-kb transcripts, increased 2.8- and 3.0-fold ($P<0.001$), respectively, when expressed per 12 μg total RNA (FIG. **5**D). When GHRH-R mRNA levels were analyzed per anterior pituitary total RNA content, levels of the 2.5-kb transcript and combined levels of 2.5-kb and 4-kb transcripts increased 1.5-fold ($P<0.05$) (data not shown). After 5 days of water deprivation, pituitary levels of the 2.5-kb GHRH-R mRNA transcript and combined levels of 2.5-kb and 4-kb transcripts, when expressed per 12 μg total RNA, increased 1.8- and 1.9-fold ($P<0.001$), respectively (data not shown). When GHRH-R mRNA levels were analyzed per anterior pituitary total RNA content, levels of the 2.5-kb transcript and combined levels of 2.5-kb and 4-kb transcripts were increased 1.3- ($P<0.05$) and 1.4-fold ($P<0.01$), respectively (data not shown).

[0137] As such, the pituitary GHRH-R, which is exclusively localized on somatotroph cells,[52] was found to be insensitive to the high-salt diet, contrarily to that of ascending thin limbs, demonstrating the vulnerability of the latter.

[0138] A tissue-specific regulation of renal medulla and anterior pituitary GHRH-R mRNA levels has previously been shown in developing and aging rat.[33] Whether or not somatotroph sensitivity to GHRH can be altered during the first 2 days of the high-NaCl diet, without affecting GHRH-R mRNA levels, remains possible. In contrast, a water deprivation strongly increased pituitary cell and tissue GHRH-R mRNA levels. Since it induces a drastic reduction of food intake and that dietary protein restriction down-regulates hypothalamic preproGHRH mRNA,[53] a subsequent decrease of pituitary GHRH-R may have occurred. These results suggest that somatotroph and ascending thin limb cell GHRH-R mRNA levels may be primarily regulated by hypothalamic and renal GHRH, respectively.

Example 5

Sensitivity to GHRH in Semi-Purified Thin Limbs of Henle's Loop Cells from Rats Submitted to a 2-, 7- or 14-Day High-NaCl Diet

[0139] Sensitivity to GHRH in thin limbs of Henle's loop cells from rats submitted to a 2-, 7- or 14-day high-NaCl diet was assessed by measuring GHRH-induced intracellular cAMP production, in freshly dispersed semi-purified thin limb cells as shown in FIG. **6**. Basal or forskolin levels of immunoreactive cAMP were not significantly decreased in rats fed 2 days with 8%-NaCl chow, although a trend was observed. Sensitivity to rGHRH(1-29)NH_2 was altered and GHRH-induced cAMP production was decreased 1.5-fold (1 nM: $P<0.01$; 100 nM: $P<0.05$) (FIG. **6**A). This loss of sensitivity to GHRH was reverted in rats fed the high-salt diet for 7 or 14 days (FIGS. **6**B, **6**C).

[0140] The regulation of GHRH-R mRNA levels was therefore reflected in the sensitivity of GHRH to induce cAMP production in freshly dispersed thin limb cells from rats submitted to the high-NaCl diet, in comparison to those fed the control diet. After 2 days of high-salt diet, a stimulation with either a low (1 nM) or high (100 nM) concentration of GHRH resulted in a decreased production of cAMP, correlating with that of GHRH-R mRNA levels. After 7 days, GHRH-induced cAMP levels were restored, indicating that an increased production of GHRH-R mRNA may be necessary to rapidly restore GHRH sensitivity and likely GHRH-R functional receptor levels. Data on the effect of high-salt-induced oxidative stress in thin limb cells specifically is unique to this study.

Example 6

In Vivo Effect of a GHRH Treatment on DNA Repair/Synthesis in Purified Thin Limbs of Henle's Loop Cells from Rats Submitted to a 2-Day High-NaCl Diet

[0141] As shown in FIG. **7**, when 2-month-old rats fed 2 days to a 8%-NaCl chow were injected daily with GHRH (1 mg/kg BW sc/day), a 5 times increase in the number of ascending thin limb cell nuclei and mitochondria immunolabeled to BrdU was observed in thin limbs of Henle's loop cells ($P<0.05$), when compared to various control groups (normal diet, with or without GHRH injections, high-salt diet alone). Moreover, the intensity of mitochondrial BrdU immunofluorescence was increased ≈7 times in these cells ($P<0.05$). GHRH-R mRNA levels tended to increase in the renal medulla of the high-NaCl fed rats, injected with GHRH and serum total insulin-like growth factor-1 (IGF-1) levels were not modified (data not shown). A subcutaneous (sc) administration of GHRH, once a day from the beginning of a 2-day high-NaCl diet, increased markedly the number of ascending thin cell nuclei and mitochondria immunolabeled to BrdU. In addition, the intensity of anti-BrdU labeling was significantly augmented in the cytoplasm co-labeling with MitoTracker red CMXRos, a reliable indicator of functional mitochondria.

[0142] Thus, in condition of oxidative stress, activation of the renal GHRH-R plays a role in adaptive processes related to DNA repair and/or synthesis, leading to cell survival and subsequent proliferation of these squamous epithelial cells not very rich in mitochondria.

Example 7

In Vivo Effect of a GHRH Treatment on GHRH-R and ClCk-1 mRNA Levels in Purified Thin Limb Cells from Rats Submitted to a 2-Day High-NaCl Diet

[0143] The in vivo effect of GHRH administration on GHRH-R and CICK-1 mRNA levels (measured by real-time RT-PCR) in purified thin limb cells from rats submitted to a 2-day high-NaCl diet was studied. GHRH-R (FIG. **8**A) and CICK-1 (FIG. **8**B) mRNA levels were significantly increased in the total renal medulla of a subgroup of 3 rats, essential in ascending thin limb functions. GHRH-R mRNA levels were decreased without significantly altering those of CIC-K1 in 5 others. Regulation of GHRH-R mRNA levels in thin limb cells of 2-month-old healthy male Sprague Dawley rats eas also studied following a subcutaneous administration of rGHRH(1-29)NH2 (0.5 mg/kg BW/day) or the saline vehicle, for 2 days. Six animals were used in each treatment. **$P<0.01$ (FIG. **8**C) These results indicate that a lower GHRH dosage, such as 0.5 mg/kg BW/day, will up-regulate the renal GHRH-R in a large number of rats.

[0144] As discussed above, GHRH directly induces thin limb cell proliferation in vitro. No significant regulatory effect was seen on anterior pituitary GHRH-R mRNA levels with a 2-day in vivo of GHRH (data not shown). It was previously shown that IGF-I serum levels are significantly decreased after a 14-day sc administration of 1 mg/kg BW/day rGHRH(1-29)NH_2 but not with 0.5 mg/kg BW/day).

[10] Therefore, the dosage and duration used to regulate the renal GHRH-R will not regulate the pituitary GHRH-R.

Example 9

In Vivo Effect of a GHRH Treatment on Cell Proliferation in Purified Thin Limb Cells from Normal Rats

[0145] The effect of GHRH on proliferation was directly assessed in semi-purified thin limbs of Henle's loop cells from healthy 2-month-old rats. As shown in FIG. **9**, rGHRH (1-29)NH_2 induced a 2.4 to 3.2-fold increase of the proliferative index in these cells (1 and 10 nM: $P<0.05$; 100 nM: $P<0.01$) when compared to control cell stimulated with the GHRH vehicle.

Example 8

RT-PCR Products from Rat and Porcine Anterior Pituitary and Renal Medulla, Using a Panel of Anterior Pituitary GHRH-R Primers

[0146] Using a panel of primers targeting the 5' end, median portion and 3' end of the pituitary GHRH-R, RT-PCR products from rat and porcine anterior pituitary and renal medulla were studied. A similar pattern was observed in both the rat and porcine renal medulla in comparison with anterior pituitary. No signal was detected in the rat (FIG. **10**A) and porcine (FIG. **10**B) medulla when 5' end primers were used.

[0147] Difference in the primary structure of the pituitary and renal GHRH-R and/or the relative abundance of the native 423-aa GHRH-R and isoforms may contribute to a tissue-specific regulation. In the rat pituitary, apart from the 423-aa GHRH-R, two splice variants have been identified[2, 11, 54] but their relative abundance has not been quantified rigorously. The 464-aa variant bears a 41-aa addition inserted into the 3rd IC domain,[11] while the 480-aa variant bears the long 3rd IC loop and a modified C-terminus, resulting from a 131-bp deletion (nt 1279-1408).[54] GHRH binds with moderate affinity to the 464-aa variant, transiently transfected in HeLa cells, and induces[55] or not[11] cAMP production. The ability of GHRH to stimulate cAMP was reported to be lower with the 480-aa variant than the 464-aa variant,[54] suggesting that the 3rd IC loop and the C-terminus are critical for GHRH-activation of the cAMP-AC-PKA pathway.

[0148] Action of GHRH is mediated in rat ascending thin limb cells by a GHRH-R exhibiting a 5' DNA sequence different from that of the rat anterior pituitary GHRH-R. This structural difference in the rat renal GHRH-R compared to the pituitary GHRH-R was also observed for the murine (data not shown) and porcine renal GHRH-R. As porcine and human pituitary GHRH-R share the highest sequence identity (86%),[2, 3] it is suggested that the renal GHRH-R variant found in the rat medulla is also present in human renal medulla.

Example 10

Identification of Renal Medulla GHRH Receptor from Rat

[0149] Considering the difference in the 5' coding region compared to that of anterior pituitary and to obtain information on the renal GHRH-R cDNA sequence, a 5' RACE was performed with the SMART RACE cDNA Amplification kit (Clontech) as described in Example 1. It allowed generating a high yield of full-length, double-stranded cDNA from small amounts of starting RNA and contains all reagents to synthesize cDNA and perform 5' and 3' amplification reactions from the same template.

[0150] Although structure difference between the renal and pituitary GHRH-R in the 3' coding region were not expected, 3' RACE was performed as a positive control. When needed, nested primers were used. Total RNA from renal medulla of 2-month-old male SD rats was extracted with TRIzol. Poly (A) mRNA was used after purification on Oligotex resin (Qiagen). The purity and integrity of total RNA was analyzed using an Agilent 2100 Bioanalyser (Agilent Technologies). The specific primers selected hybridized to the central region of the pituitary receptor cDNA and exhibit the various physico-chemical characteristics required in this approach. The amplified sequences in the 5' and 3' RACE strategies overlaped each other in a tract of 172 nucleotides, to facilitate sequence analysis.

[0151] Amplification products were resolved on agarose gel electrophoresis and isolated with NucleoTrap Gel Extraction kit, provided with the SMART RACE system, quantified and quality-controlled by electrophoresis. Since the proofreading activity of the polymerase removes the 3' overhangs during PCR and makes TA cloning very inefficient, fragments were treated with Taq Polymerase, after gel extraction to add the 3'A overhangs necessary for the TA cloning. DNA fragments were directly cloned into the pCR4-TOPO vector (Invitrogen) and the vector was amplified using *E. coli* Top10 (Invitrogen). Cloned inserts were sequenced.

[0152] The 5' RACE gave three products of 1.1, 0.9 and 0.8 kb from renal medulla Poly(A)$^+$RNA, using UPM/Ex-9/10P and NUP/Ex-9P primer pairs (FIG. **11**A, lane 1). A single product of 1.0 kb (expected size: 0.93 kb) was obtained from anterior pituitary Poly(A)$^+$RNA (positive control) (FIG. **11**A, lane 2), and no signal was obtained with liver RNA (negative control) (FIG. **2**A, lane 3). Following purification (FIG. **11**A, lanes 4-7), insertion of individual products in the plasmid pCR4—TOPO, transformation of TOP10 *E. coli* colonies, and plasmid amplification, 4, 7 and 10 sequences were analyzed for the 1.1-, 0.9- and 0.8-kb renal GHRH-R cDNA fragments, respectively, as well as three sequences for the 1.0-kb pituitary GHRH-R cDNA fragment.

[0153] The 3' RACE gave two major products of 1.2 and 0.9 kb (expected size: 0.92 kb), exhibiting no apparent size difference between renal medulla and anterior pituitary Poly (A)$^+$RNA (FIG. **11**B, lanes 1-2). The size of low intensity products obtained using liver RNA was not compatible with that of a GHRH-R entity (FIG. **11**B, lane 3). Each renal medulla and anterior pituitary PCR product obtained was homogeneous after gel extraction (FIG. **11**B, lanes 4-7), and four sequences were obtained for each one.

[0154] GHRH-R Sequence Analysis

[0155] When the renal medulla 5'-RACE 1.1-kb product was aligned with 3'-RACE 1.2-kb renal/pituitary product, the resulting GHRH-R cDNA sequence was homologous with that of anterior pituitary, from the beginning of exon 2 to the stop codon. Upstream of exon 2, the renal medulla GHRH-R sequence did not match that of anterior pituitary GHRH-R exon 1 or intron 1, excluding the possibility of alternative splicing in intron 1. The 306-bp novel sequence, corresponding to a different region on chromosome 4, was located at approximately 19,000 bp upstream of the anterior pituitary GHRH-R transcription start site (FIG. **12**). When compared to the genomic DNA sequence (AC091710), it was shown to contain two exons, with splice donors and acceptors in intronic sequences (FIG. **12**). They were named exon 1M and 2M for the renal medulla GHRH-R exon 1 and 2, respectively. When the anterior pituitary 5'-RACE 1.0-kb product was aligned with 3'-RACE 1.2-kb renal/pituitary product, the resulting GHRH-R cDNA sequence was completely homologous with the 1269-bp pituitary sequence. The renal medulla and anterior pituitary GHRH-R cDNAs contained all splice donor and acceptor sequences to form a 14- and 13-exon sequence, respectively (FIG. **12**). The osition of exons of the anterior pituitary and renal GHRH-R on the genomic sequence AC091710 is shown in Table 4.

TABLE 4

Position of exons of the anterior pituitary and renal GHRH-R on the genomic sequence AC091710

	Anterior pituitary sequence	Renal medulla sequence
Exon A/Exon 1M		18576-18768
Exon B/Exon 2M		19299-19411
Exon 1	38055-38138	
Exon 2/Exon 3M	40720-40822	40720-40822
Exon 3/Exon 4M	40933-41040	40933-41040
Exon 4/Exon 5M	41592-41689	41592-41689
Exon 5/Exon 6M	42686-42783	42686-42783
Exon 6/Exon 7M	43582-43714	43582-43714
Exon 7/Exon 8M	45040-45193	45040-45193
Exon 8/Exon 9M	45480-45540	45480-45540
Exon 9/Exon 10M	45959-46028	45959-46028
Exon 10/Exon 11M	46594-46685	46594-46685
Exon 11/Exon 12M	47226-47355	47226-47355
Exon 12/Exon 13M	48709-48750	48709-48750
Exon 13/Exon 14M	50577-51033	50577-51033

[0156] The loss of the pituitary exon 1 in the renal GHRH-R cDNA sequence leads to a loss of the initiation of translation codon described in the anterior pituitary GHRH-R sequence. No start ATG was found in exon 1M or 2M. However, a Kozak consensus-like sequence (tgctccATGG instead of gcca/gccATGG) is present in exon 3M (exon 2 in the pituitary GHRH-R sequence), with a 1113-bp open reading frame (ORF). This ORF will lead to a 371-aa deduced protein instead of the 423-aa anterior pituitary GHRH-R, identical to the portion 53-423 of the pituitary GHRH-R and lacking the first 52 aa. This aa sequence retains the seven TM domains, five out of the six Cys conserved in the 423-aa GHRH-R sequence, and a crucial Asp in position 8 (Asp^{60} in the pituitary GHRH-R). However the renal GHRH-R would lack the Asn in position 50 of the pituitary GHRH-R, a site for potential glycosylation.

[0157] Sequencing of the renal medulla 5'-RACE 0.9-kb product (FIG. **11**A, lanes 1, 5) revealed the presence of two entities. The first one exhibited the same structure than the 1.1-kb product described above, while lacking exon 8M (pituitary GHRH-R exon 7). The absence of exon 8M, due to alternative splicing would change the ORF, leading to the insertion of a premature stop codon in exon 10M. The putative 171-aa truncated GHRH-R (aa sequence 53-199 of the pituitary GHRH-R) would only have four TM. The last 24 aa of this truncated receptor having no homology with the pituitary GHRH-R. The second product corresponded to the 1.1-kb sequence, but lacking exon 1M. Interestingly, it would lead to the 371-aa GHRH-R described above, with the start codon in exon 3M.

[0158] Analysis of the renal medulla 0.8-kb 5'-RACE product (FIG. **11**A, lanes 1, 6), led to the identification of three GHRH-R cDNA sequences. The first and second one began with exon 3M and 4M, respectively. The deduced aa sequences generated would be the 371-aa GHRH-R already described and a 227-aa product (aa sequence 197-423 of the pituitary GHRH-R), respectively. Finally, the first two exons of the third sequence had no homology with either the anterior pituitary GHRH-R exon 1 or the renal GHRH-R exon 1M or 2M. This sequence corresponded to a genomic sequence, located at approximately 15,500 bp upstream of the pituitary initiation transcription site on chromosome 4. When this sequence was present, exons 1M to 6M and 8M were absent, leading to a putative 33-aa peptide devoid of homology with any known protein from the rat genome.

[0159] The 21 sequences obtained from the 5'-RACE products indicates that at least 52% of the cDNA sequences (11/21) would lead to the 371-aa GHRH-R, while 14% and 19% could generate a 227- and 171-aa GHRH-R, respectively. In addition to structure differences reported, the renal GHRH-R transcribed exhibited a long 5' UTR (405 nt) contrarily to that of the pituitary receptor which is very short (27 nt).

[0160] The majority of the GHRH-R cDNA sequences found in renal medulla led to a unique amino acid sequence identical to the portion 53-423 of the 423-aa rat anterior pituitary GHRH-R. The nucleic acid sequence of rat renal medulla GHRH receptor is shown in FIG. **13** and its deduced protein sequence is found in FIG. **14**. A comparison of the GHRH-R protein sequence between anterior pituitary and renal medulla is found in FIG. **15**.

[0161] Detection of Renal GHRH-R RT-PCR Products in Purified Rat Thin Limb Henle's Loop Cells and Anterior Pituitary

[0162] The presence of exons 1M and 2M was validated in tlHL cells of the renal medulla as this cell type contains the highest level of GHRH-R in renal tissue and anterior pituitary. PCR amplification of total RNA from tlHL cells was performed using primers targeting exon 1M and 11M (exon 10 in the pituitary) or exon 2M and 11M (Table 2, FIG. **16**A). The size of PCR products obtained using primer pairs Ex-1M/Ex-11M (FIG. **16**A, lane 1) and Ex-2M/Ex-11M (FIG. **16**A, lane 4) was of 1.10 and 0.97 kb, respectively (expected size: 1.10 and 0.94 kb). No specific amplification was observed with anterior pituitary (FIG. **16**A, lanes 2, 5) and liver (FIG. **16**A, lanes 3, 6) total RNA. Therefore, the novel cDNA sequences identified in the renal medulla GHRH-R, with exons 1M and 2M shows a tissue-selectivity. The presence of a 0.8-kb product (FIG. **16**, lane 4) when the primer pair Ex 2M/Ex 11M was used could be compatible with the presence of a cDNA lacking exon 8M (expected size: 0.79 kb). When the primer pair Ex 6P/Ex 11M was used, a strong signal was obtained (0.40 kb; expected size: 0.37 kb) both with renal (FIG. **16**B, lane 1) and pituitary RNA (FIG. **16**B, lane 2), indicating that the presence of an entity lacking exon 8M (exon 7 in the pituitary) is likely very low in tlHL and absent in anterior pituitary. No PCR product was detected in liver RNA with primers Ex 6P/Ex 11M (FIG. **16**B, lane 3).

[0163] Characterization of the Renal Medulla and Anterior Pituitary 5' Upstream Untranscribed Region

[0164] Using Transcription Element Search System (TESS) tools, regions 16601-18600 and 36080-38079 upstream to the transcription initiation site of the renal and anterior pituitary GHRH-R, respectively, were analyzed. As shown in Tables 5 and 6, putative binding sites for several known transcription factors were identified. The most abundant number of sites for transcription factors, found both in the anterior pituitary and renal 5' untranscribed GHRH-R region (Table 5), were: glucocorticoid receptor (GR), Pit 1 (POU1F1a), upstream stimulatory factors 1 and 2 (USF and USF2), and Brain-2 (POU3F2). The most abundant and specific binding sites to the renal 5' untranscribed GHRH-R region, was F2F (Table 6). Most of putative pituitary transcription binding sites found in 2 kb upstream the anterior pituitary GHRH-R cDNA transcription start site, were also present in this 2-kb cDNA renal medulla sequence, such as CREB (cAMP response element binding-protein), GR (glucocorticoid receptor), POU1F1a (Pit-1) and T3R-alpha (thyroid hormone receptor). Among common binding sites, Pit-1 and GR was well known to stimulate GHRH-R expression in pituitary. But in renal medulla this same regulator won't lead to pituitary GHRH-R expression. This situation suggests that GHRH-R pituitary promoter is not accessible to the binding of this transcription factor, on tlHL cells. In addition, several binding sites are unique to GHRH-R renal upstream region compared to GHRH-R pituitary upstream region. These different factors may be responsible to the GHRH-R tissue-specific expression in renal medulla. The most abundant binding site was found for the F2F transcription factor. F2F are involved principally in gene repression in non pituitary cells. In kidney, F2F may repress GHRH-R expression in non tlHL cells to keep GHRH-R expression specific to tlHL.

TABLE 5

Putative transcription factor binding sites found in the 5' untranscribed region of renal medulla GHRH-R and anterior pituitary

Name	Number of sites in 5'untranscribed region of the renal medulla GHRH-R (region 16601-18600)	Number of sites in 5'-untranscribed region of the anterior pituitary GHRH-R (region 36080-38079)
Glucocorticoid receptor (GR)	19	18
Pit-1 (POU1F1a)	10	3
Upstream stimulatory factors (USF)	9	3
Upstream stimulatory factors-2 (USF2)	7	3
Brain-2 (POU3F2)	7	3
CCAAT/enhancer binding protein α (C/EBP α)	6	4
Pan-2(E12)	4	7
Interleukin-6 response element-binding protein (IL-6 RE-BP)	4	7
Thyroïd hormone receptor a (T3R-α)	4	4
c-Jun	4	2
Enhancer-binding protein-45 (EBP-45)	4	2
PEA3	3	3
c-Fos	3	2
GATA-4	3	2
Nuclear factor-1 (NF1)	3	2
Nuclear factor BA1 (NF-BA1)	3	1
Hepatocyte nuclear factor α (HNF-1)	2	3
Myogenin	2	3
Pancreatic transcription factor 1 (PTF-1)	2	3
δ-Crystallin enhancer factor 1 (δEF1)	2	2
Nerve growth factor induced-B (NGFI-B__	2	2
Peroxisome proliferator-activated receptor α (PPAR-α)	2	2
Snail	2	2
cAMP response element binding-protein (CREB)	2	1
Neuronal stem cell leukemia (NSCL-1)	2	1
Specificity protein 1 (Sp1)	1	3
Activator Protein-1 (AP-1)	1	2
Androgen receptor (AR)	1	2
Cone rod homeobox protein (crx)	1	2
Hepatocyte nuclear factor 3 (HNF-3α)	1	2
Hepatocyte nuclear factor-3B (HNF-3B)	1	1
Hepatocyte nuclear factor-3γ (HNF-3γ)	1	1

TABLE 6

List of putative transcription factor binding sites specific to the 5'-untranscribed region of renal medulla or anterior pituitary GHRH-R

	Name	Number of sites in 5'un-transcribed region of the renal medulla GHRH-R (region 16601-18600)
Example	(F2F)	7
11	Transcription factor-IID	3
Identificat	(TFIID)	
	c-Myc	2
	Hepatocyte nuclear factor-1B (HNF-1B)	2
	Aryl-hydrocarbon receptor nuclear translocator (Arnt)	1
	basic leucine zipper (bZIP)	1
	c-Myb	1
	chicken ovalbumin upstream promoter-I (COUP)	1
	Delta CREB; CREB-A (variant d'épissage de CREB)	1
	Enhancer-binding protein-40 (EBP-40)	1
	GA binding protein	1
	GABP	1
	Homeobox	1
	lymphoid enhancing factor 1 (LEF-1)	1
	Activating transcription factor 3 (ATF-3)	1
	Mammalian achaete-scute homolog 1 (MASH-1)	1
	Mammalian achaete-scute homolog 1 (MASH-2)	1

TABLE 6-continued

List of putative transcription factor binding sites specific to the 5'-untranscribed region of renal medulla or anterior pituitary GHRH-R

Muscle regulatory factor-4 (MRF4)	1
Mycn	1

Name	Number of sites in 5'un-transcribed region of the anterior pituitary GHRH-R (region 36080-38079)
Sox5	2
Zinc finger-42_1-4 (ZNF42_1-4)	2
CCAAT-binding factor (CBP)	1
D-binding protein (DBP)	1
Nuclear factor-1/L (NF-1/L)	1
sis-inducing factor (SIF)	1
translation elongation factor-2 (TEF2)	1

[0165] Identification of the 5' and 3' cDNA sequence of the GHRH-R from human renal medullawas performed using the same strategy used for the rat GHRH-R(SMART RACE cDNA Amplification kit (Clontech). Poly (A) RNA from human renal medulla and pituitary was used along with the following primer sequences: B1, 3' RACE primer: Positions 546 to 573 of the human GHRH-R sequence in Genbank (AY557192) $B_{1:}$ $_5$'-GGA TGC TGC CCT TTT CCA CAG CGA CGA C-3'; B2, 5' RACE primer: Positions 684 to 706 of the human GHRH-R sequence. B_2: 5'-GGG AGG TGG AGG CCA GGA GGC AG-3'; C1, 3' Nested RACE primer: Positions 570 to 597 of the human GHRH-R sequence in Genbank (AY557192) C_1: 5'-CGA CAC TGA CCA CTG CAG CTT CTC CAC T-3'; C2, 5' Nested RACE primer: Positions 671 to 697 of the human GHRH-R sequence. C_2: 5'-AGG CCA GGA GGC AGT TCA GGT AGA CGG-3'.

[0166] Amplified sequences in the 5' and 3' RACE strategies overlaped each other in a tract of 161 nucleotides to facilitate sequence analysis. To document the relative abundance of human renal GHRH-R variants, RT-PCR was performed using several specific primers from different exons. The post-mortem renal medullas and pituitaries were obtained from two different men, dead from massive heart attack in their fifties (Quebec-Transplant, Montreal via Dr J Tremblay and the University of St-Jacques de Compostela, Spain via Dr T Garcia-Caballero).

[0167] Two main sequences encoding respectively a 400 amino acids (SEQ ID NO.: 16) and a 416 amino acids (SEQ ID NO.: 17) protein were identified, with the 400 amino acids sequence being the most abundant. The 400 amino acids protein corresponds to amino acids 24 to 425 of human pituitary GHRH receptor. The 416 amino acids protein corresponds to amino acids 54-423 of human pituitary GHRH receptor with the first 46 amino acids (N-terminus) being unique to the human renal form of GHRH receptor.

[0168] The nucleic acid sequence of human renal medulla GHRH receptor is shown in FIG. **17** and its deduced protein sequence is found in FIG. **18**. A comparison of the GHRH-R protein sequence between anterior pituitary and renal medulla is found in FIG. **19**.

[0169] The position of exons of the human anterior pituitary and human renal GHRH-R on chromosome 7 genomic sequence is shown in Table 7.

TABLE 7

Position of exons of the human anterior pituitary and renal GHRH-R on chromosome 7 genomic sequence

	Anterior pituitary sequence	Renal medulla sequence (400 aa)	Renal medulla sequence (416 aa)
Exon 1	30970209-30970265	30974797-30974848	—
Exon 2	30974974-30975076	30974971-30975076	30974782-30974973
Exon 3	30975203-30975310	30975203-30975310	30975131-30975310
Exon 4	30976007-30976104	30976007-30976104	30976007-30976104
Exon 5	30977259-30977356	30977259-30977356	30977259-30977356
Exon 6	30978103-30978235	30978103-30978235	30978103-30978236
Exon 7	30980125-30980278	30980125-30980278	30980126-30980278
Exon 8	30980569-30980629	30980569-30980629	30980569-30980629
Exon 9	30981111-30981180	30981111-30981180	30981111-30981180
Exon 10	30981917-30982008	30981917-30982008	30981917-30982008
Exon 11	30982569-30982698	30982569-30982698	30982569-30982698
Exon 12	30983421-30983462	30983421-30983462	30983421-30983462
Exon 13	30985259-30985384	30985259-30985668	30985259-30985668

[0170] In the present study, regulation of renal and pituitary GHRH-R mRNA levels was examined using in vivo models of NaCl or water homeostasis disruption. The presence of a unique GHRH/GHRH-R system in Henle's loop ascending thin limb cell and the specific regulation of GHRH-R mRNA levels and GHRH sensitivity in a situation of hyperosmotic stress, together with the strong effect of GHRH on mitochondrial and nuclear DNA repair/synthesis, indicate a role for GHRH in renoprotection. GHRH appears to be involved in adaptive processes related to DNA repair and/or synthesis thereby protecting ascending limb cell function in subjects with renal vulnerability (aging, diabetes) and were a health event could lead to a production of oxidative stress (antibiotic toxicity, cancer chemotherapeutic agent toxicity, infection, inflammation, ischemia) and renal failure. The Applicant has come to the unexpected discovery that the GHRH receptor (GHRH-R) expressed in kidney cells is different than the pituitary GHRH receptor. The renal GHRH-R is biologically active even if it does not contain the first 52 amino acids and/or the first 80 amino acids of the pituitary GHRH-R.

[0171] Although the present invention has been described by way of exemplary embodiments, it should be understood by those skilled in the art that the foregoing and various other changes, omission and additions may be made therein and thereto, without departing from the spirit and scope of the present invention as defined in the appended claims.

SEQUENCE LISTING

SEQ ID NO.: 1-

GHRH(1-44)
Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln Leu Ser Ala Arg Lys

Leu Leu Gln Asp Ile Met Ser Arg Gln Gln Gly Glu Ser Asn Gln Glu Arg Gly Ala Arg Ala

Arg Leu

-continued

SEQ ID NO. 2:
Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln Leu Ser Ala Arg Lys

Leu Leu Gln Asp Ile Met Ser Arg-Xaa
Wherein Xaa is either absent or any amino acid sequence of 1 up to 15 residues

SEQ ID NO.: 3-
GHRH(1-29)
Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln Leu Ser Ala Arg Lys

Leu Leu Gln Asp Ile Met Ser Arg

SEQ ID NO.: 4
Tyr-D-Ala^{2}-Asp-Ala-Ile-Phe-Thr-Ala-Ser-Tyr-Arg-Lys-Val-Leu-Ala-Gln-Leu-Ser-Ala-Arg-

Lys-Lys-Leu-Gln-Asp-Ile-Met-Ser-Arg-Xaa,
wherein Xaa is either absent or any amino acid sequence of 1 up to 15 residues

SEQ ID NO.: 5
Tyr-D-Ala^{2}-Asp-Ala-Ile-Phe-Thr-Asn-Ser-D-Tyr^{10}-Arg-Lys-Val-Leu-Gly-Gln-Leu-Ser-Ala-

Arg-Lys-Lys-Leu-Gln-Asp-Ile-Met-Ser-Arg-Xaa
wherein Xaa is either absent or any amino acid sequence of 1 up to 15 residues

SEQ ID NO.: 6
Tyr- D-Ala^{2}-Asp-Ala-Ile-Phe-Thr-Asn-Ser-D-Tyr^{10}-Arg-Lys-Val-Leu-D-Ala^{15}-Gln-Leu-Ser-

Ala-Arg-Lys-Lys^{22}-Leu-Gln-Asp-Ile-Met-Ser-Arg-Xaa
wherein Xaa is either absent or any amino acid sequence of 1 up to 15 residues

SEQ ID NO.: 7
Tyr Ala Asp Ala Ile Phe Thr Ala^{8} Ser Tyr Arg Lys Val Leu Ala^{15} Gln Leu Ser Ala Arg Lys

Ala^{22} Leu Gln Asp Ile Met Ser Arg

SEQ ID NO.: 8
Tyr Ala Asp Ala Ile Phe Thr Ala^{8} Ala^{9} Tyr Arg Lys Val Leu Ala^{15} Gln Leu Ser Ala Arg Lys

Ala^{22} Leu Gln Asp Ile Met Ser Arg

SEQ ID NO.: 9
Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln Leu Ser Ala Arg Lys

Lys^{22} Leu Gln Asp Ile Met Ser Arg

SEQ ID NO.: 10
Tyr-Xaa2-Asp-Ala-Ile-Phe-Thr-Xaa8-Xaa9-Xaa10-Arg-Lys-Val-Leu-Xaa15-Gln-Leu-Ser-

Ala-Arg-Xaa21-Xaa22-Leu-Gln-Asp-Ile-Met-Ser-Arg-Xaa30,
wherein:

Xaa2 is Ala or D-Ala;

Xaa 8 is Asn, D-Asn or Ala;

Xaa 9 is Ser or Ala;

Xaa 10 is Tyr or D-Tyr;

Xaa 15 is Gly, Ala or D-Ala;

Xaa 21 is Lys or D-Lys;

Xaa 22 is Leu, D-Leu, Lys or Ala; and

Xaa 30 is a bond or any amino acid sequence of 1 up to 15 residues and wherein the analogue comprises at least one of the above amino acid substitution in comparison with the amino acid sequence of the native form of hGHRH1-29.

-continued

SEQ ID NO.: 11

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<223> nucleotide varying in comparison to the genomic sequence

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gatcctgaga gctccagtgt ccacttcttg ccatccttct gtatgagtgg gaaaccaacc 180

caggccaccc ttgggggcag gtcctgggac ttcttggagc agatgagcct ttatccatgc 240

gacagaacag gaggtaccta ctgacaccca tgtcctagga tagacacatc tgtatgtggg 300

tgcgaggcac tgggtcacct ccacctagaa tgtgacttca tcactcagct gagagacgat 360

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cactgcccca ggaactacat ccacacgcag ctgtttgcta ctttcatcct caaggccagt 780

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SEQ ID NO.: 13

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Gly Ser Gly Gln Trp Val Ser Leu Pro Cys Pro Glu Phe Phe Ser His
20 25 30

Phe Gly Ser Asp Pro Gly Ala Val Lys Arg Asp Cys Thr Ile Thr Gly
35 40 45

Trp Ser Asp Pro Phe Pro Pro Tyr Pro Val Ala Cys Pro Val Pro Leu
50 55 60

Glu Leu Leu Thr Glu Glu Lys Ser Tyr Phe Ser Thr Val Lys Ile Ile
65 70 75 80

Tyr Thr Thr Gly His Ser Ile Ser Ile Val Ala Leu Cys Val Ala Ile
85 90 95

Ala Ile Leu Val Ala Leu Arg Arg Leu His Cys Pro Arg Asn Tyr Ile

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```
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        115                 120                 125

Leu Lys Asp Ala Ala Val Phe Gln Gly Asp Ser Thr Asp His Cys Ser
    130                 135                 140

Met Ser Thr Ile Leu Cys Lys Val Ser Val Ala Val Ser His Phe Ala
145                 150                 155             160

Thr Met Thr Asn Phe Ser Trp Leu Leu Ala Glu Ala Val Tyr Leu Ser
                165                 170             175

Cys Leu Leu Ala Ser Thr Ser Pro Arg Ser Lys Pro Ala Phe Trp Trp
            180                 185             190

Leu Val Leu Ala Gly Trp Gly Leu Pro Val Leu Cys Thr Gly Thr Trp
        195                 200             205

Val Gly Cys Lys Leu Ala Phe Glu Asp Thr Ala Cys Trp Asp Leu Asp
    210                 215             220

Asp Ser Ser Pro Tyr Trp Trp Ile Ile Lys Gly Pro Ile Val Leu Ser
225                 230             235         240

Val Gly Val Asn Phe Gly Leu Phe Leu Asn Ile Ile Cys Ile Leu Leu
                245             250         255

Arg Lys Leu Gly Pro Ala Gln Gly Gly Leu His Thr Arg Ala Gln Tyr
            260             265         270

Trp Arg Leu Ser Lys Ser Thr Leu Leu Leu Ile Pro Leu Phe Gly Ile
        275             280         285

His Tyr Ile Ile Phe Asn Phe Leu Pro Asp Ser Ala Gly Leu Gly Ile
    290             295         300

Arg Leu Pro Leu Glu Leu Gly Leu Gly Ser Phe Gln Gly Phe Val Val
305             310         315         320

Ala Val Leu Tyr Cys Phe Leu Asn Gln Glu Val Arg Thr Glu Ile Ser
            325         330         335

Arg Lys Trp Tyr Gly His Asp Pro Glu Leu Leu Pro Ala Arg Arg Thr
        340         345         350

Cys Thr Glu Trp Thr Thr Pro Pro Arg Ser Arg Val Lys Val Leu Thr
    355         360         365

Ser Glu Cys
370
```

SEQ ID NO.: 14

52 first amino acids of AAD26335.1 or NP_036982.1 (*Rattus norvegicus*)
MDSLLWATWV LCLLNLWGVA LGHLHLECDF ITQLRDDELA CLQAAEGTNN SS

SEQ ID NO.: 15

80 first amino acids of EDL88088.1 or EDL88086.1 (*Rattus norvegicus*)
MQLGGIARDS LGGKGHGSLG RTTGTTAMDS LLWATWVLCL LNLWGVALGH

LHLECDFITQ LRDDELACLQ AAEGTNNSS

SEQ ID NO.: 16

Human renal GHRH-R (400aa)
M H P E C D F I T Q L R E D E S A C L Q A A E E M P N T T L

G C P A T W D G L L C W P T A G S G E W V T L P C P D F F S

H F S S E S G A V K R D C T I T G W S E P F P P Y P V A C P

V P L E L L A E E E S Y F S T V K I I Y T V G H S I S I V A

L F V A I T I L V A L R R L H C P R N Y V H T Q L F T T F I

L K A G A V F L K D A A L F H S D D T D H C S F S T V L C K

-continued

V S V A A S H F A T M T N F S W L L A E A V Y L N C L L A S

T S P S S R R A F W W L V L A G W G L P V L F T G T W V S C

K L A F E D I A C W D L D D T S P Y W W I I K G P I V L S V

G V N F G L F L N I I R I L V R K L E P A Q G S L H T Q S Q

Y W R L S K S T L F L I P L F G I H Y I I F N F L P D N A G

L G I R L P L E L G L G S F Q G F I V A I L Y C F L N Q E V

R T E I S R K W H G H D P E L L P A W R T R A K W T T P S R

S A A K V L T S M C

SEQ ID NO.: 17

Human renal GHRH-R (416aa)

M W T T E P R K T P K W L G S S C S L F P A A S P L G L L G

Q P C T W A E S L L L L A L Y P G C P A T W D G L L C W P T

A G S G E W V T L P C P D F F S H F S S E S G A V K R D C T

I T G W S E P F P P Y P V A C P V P L E L L A E E E S Y F S

T V K I I Y T V G H S I S I V A L F V A I T I L V A L R R L

H C P R N Y V H T Q L F T T F I L K A G A V F L K D A A L F

H S D D T D H C S F S T V L C K V S V A A S H F A T M T N F

S W L L A E A V Y L N C L L A S T S P S S R R A F W W L V L

A G W G L P V L F T G T W V S C K L A F E D I A C W D L D D

T S P Y W W I I K G P I V L S V G V N F G L F L N I I R I L

V R K L E P A Q G S L H T Q S Q Y W R L S K S T L F L I P L

F G I H Y I I F N F L P D N A G L G I R L P L E L G L G S F

Q G F I V A I L Y C F L N Q E V R T E I S R K W H G H D P E

L L P A W R T R A K W T T P S R S A A K V L T S M C

SEQ ID NO.: 18

Human GHRH-R 400 total nuc

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gtccctgtatgtctacctctgacttctgtggtccctctgtgtctgctctcatccattcctcttactggggcatggggctctagcccaa

ggctcagaggagccaataaacctgtaaatgaaaaaaaaaaaaa

SEQ ID NO.: 19

Human GHRH-R 400 start nuc
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SEQ ID NO.: 20

Human GHRH-R 416 total nuc
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ccattcctcttactggggcatggggctctagcccaaggctcagaggagccaataaacctgtaaatgaaaaaaaaaaaa

SEQ ID NO.: 21

Human GHRH-R 416 start nuc
atgtggaccacagagcccagaaagacacccaaatggcttggctcatcctgttcactgttccagcagcctcacccctcgga

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tag

SEQ ID NO.: 22

23 first amino acids of growth hormone-releasing hormone receptor *Homo sapiens* AAS59864 gi: 45386101
mdrrmwgahv fcvlsplptv lgh

SEQ ID NO.: 23

53 first amino acids of growth hormone-releasing hormone receptor *Homo sapiens* AAS59864 gi: 45386101
mdrrmwgahv fcvlsplptv lghmhpecdf itqlredesa clqaaeempn ttl

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SEQUENCE LISTING

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<160> NUMBER OF SEQ ID NOS: 132

<210> SEQ ID NO 1
<211> LENGTH: 44
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

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Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg Gln Gln Gly
            20                  25                  30

Glu Ser Asn Gln Glu Arg Gly Ala Arg Ala Arg Leu
        35                  40


<210> SEQ ID NO 2
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<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 2

Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg
            20                  25


<210> SEQ ID NO 3
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(30)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 3

Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg Xaa
            20                  25                  30


<210> SEQ ID NO 4
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(31)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 4

Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg Xaa Xaa
            20                  25                  30


<210> SEQ ID NO 5
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(32)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 5

Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30


<210> SEQ ID NO 6
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<212> TYPE: PRT
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<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(33)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 6

Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa


<210> SEQ ID NO 7
<211> LENGTH: 34
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(34)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 7

Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa


<210> SEQ ID NO 8
<211> LENGTH: 35
<212> TYPE: PRT
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<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
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<222> LOCATION: (30)..(35)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 8

Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa
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<210> SEQ ID NO 9
<211> LENGTH: 36
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
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<222> LOCATION: (30)..(36)
<223> OTHER INFORMATION: Xaa is any amino acid
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<400> SEQUENCE: 9

Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa
        35


<210> SEQ ID NO 10
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<212> TYPE: PRT
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<220> FEATURE:
<223> OTHER INFORMATION: Chemically Syntheized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(37)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 10

Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa
        35


<210> SEQ ID NO 11
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<212> TYPE: PRT
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<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(38)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 11

Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa
        35


<210> SEQ ID NO 12
<211> LENGTH: 39
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(39)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 12

Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
```

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            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35


<210> SEQ ID NO 13
<211> LENGTH: 40
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(40)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 13

Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35                  40


<210> SEQ ID NO 14
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<212> TYPE: PRT
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<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(41)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 14

Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35                  40


<210> SEQ ID NO 15
<211> LENGTH: 42
<212> TYPE: PRT
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<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(42)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 15

Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35                  40


<210> SEQ ID NO 16
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<211> LENGTH: 43
<212> TYPE: PRT
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<221> NAME/KEY: MISC_FEATURE
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<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 16

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Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35                  40
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<210> SEQ ID NO 17
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<212> TYPE: PRT
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<223> OTHER INFORMATION: Chemically Synthesized
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<222> LOCATION: (30)..(44)
<223> OTHER INFORMATION: Xaa is any amino acid
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<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(44)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 17

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Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35                  40
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<210> SEQ ID NO 18
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthe sized

<400> SEQUENCE: 18

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Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Leu Leu Gln Asp Ile Met Ser Arg
            20                  25
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<210> SEQ ID NO 19
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<223> OTHER INFORMATION: Xaa is D-Ala

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<400> SEQUENCE: 19

Tyr Xaa Asp Ala Ile Phe Thr Ala Ser Tyr Arg Lys Val Leu Ala Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg
            20                  25


<210> SEQ ID NO 20
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<212> TYPE: PRT
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<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
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<222> LOCATION: (30)..(30)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 20

Tyr Xaa Asp Ala Ile Phe Thr Ala Ser Tyr Arg Lys Val Leu Ala Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa
            20                  25                  30


<210> SEQ ID NO 21
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<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
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<222> LOCATION: (30)..(31)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 21

Tyr Xaa Asp Ala Ile Phe Thr Ala Ser Tyr Arg Lys Val Leu Ala Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa
            20                  25                  30


<210> SEQ ID NO 22
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<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
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<222> LOCATION: (30)..(32)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 22

Tyr Xaa Asp Ala Ile Phe Thr Ala Ser Tyr Arg Lys Val Leu Ala Gln
1               5                   10                  15
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Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30


<210> SEQ ID NO 23
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<212> TYPE: PRT
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<220> FEATURE:
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<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(33)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 23

Tyr Xaa Asp Ala Ile Phe Thr Ala Ser Tyr Arg Lys Val Leu Ala Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa


<210> SEQ ID NO 24
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<220> FEATURE:
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<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(34)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 24

Tyr Xaa Asp Ala Ile Phe Thr Ala Ser Tyr Arg Lys Val Leu Ala Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa


<210> SEQ ID NO 25
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<212> TYPE: PRT
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<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(35)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 25

Tyr Xaa Asp Ala Ile Phe Thr Ala Ser Tyr Arg Lys Val Leu Ala Gln
1               5                   10                  15
```

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```
Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa
        35


<210> SEQ ID NO 26
<211> LENGTH: 36
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<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(36)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 26

Tyr Xaa Asp Ala Ile Phe Thr Ala Ser Tyr Arg Lys Val Leu Ala Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa
        35


<210> SEQ ID NO 27
<211> LENGTH: 37
<212> TYPE: PRT
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<220> FEATURE:
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<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(37)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 27

Tyr Xaa Asp Ala Ile Phe Thr Ala Ser Tyr Arg Lys Val Leu Ala Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa
        35


<210> SEQ ID NO 28
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
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<220> FEATURE:
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<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(38)
<223> OTHER INFORMATION: Xaa is any amino acid
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<400> SEQUENCE: 28

Tyr Xaa Asp Ala Ile Phe Thr Ala Ser Tyr Arg Lys Val Leu Ala Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa
        35


<210> SEQ ID NO 29
<211> LENGTH: 39
<212> TYPE: PRT
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<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
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<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(39)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 29

Tyr Xaa Asp Ala Ile Phe Thr Ala Ser Tyr Arg Lys Val Leu Ala Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa
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<210> SEQ ID NO 30
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<212> TYPE: PRT
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<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
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<222> LOCATION: (30)..(40)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 30

Tyr Xaa Asp Ala Ile Phe Thr Ala Ser Tyr Arg Lys Val Leu Ala Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35                  40


<210> SEQ ID NO 31
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<212> TYPE: PRT
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<220> FEATURE:
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<222> LOCATION: (2)..(2)
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<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(41)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 31

Tyr Xaa Asp Ala Ile Phe Thr Ala Ser Tyr Arg Lys Val Leu Ala Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35                  40


<210> SEQ ID NO 32
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
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<220> FEATURE:
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<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(42)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 32

Tyr Xaa Asp Ala Ile Phe Thr Ala Ser Tyr Arg Lys Val Leu Ala Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35                  40


<210> SEQ ID NO 33
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<212> TYPE: PRT
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<223> OTHER INFORMATION: Chemically Synthesized
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<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(43)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 33

Tyr Xaa Asp Ala Ile Phe Thr Ala Ser Tyr Arg Lys Val Leu Ala Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35                  40


<210> SEQ ID NO 34
<211> LENGTH: 44
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
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<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(44)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 34

Tyr Xaa Asp Ala Ile Phe Thr Ala Ser Tyr Arg Lys Val Leu Ala Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35                  40


<210> SEQ ID NO 35
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
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<220> FEATURE:
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<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is D-Tyr

<400> SEQUENCE: 35

Tyr Xaa Asp Ala Ile Phe Thr Asn Ser Xaa Arg Lys Val Leu Gly Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg
            20                  25


<210> SEQ ID NO 36
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(30)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 36

Tyr Xaa Asp Ala Ile Phe Thr Asn Ser Xaa Arg Lys Val Leu Gly Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa
            20                  25                  30


<210> SEQ ID NO 37
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<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(31)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 37

Tyr Xaa Asp Ala Ile Phe Thr Asn Ser Xaa Arg Lys Val Leu Gly Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa
            20                  25                  30


<210> SEQ ID NO 38
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(32)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 38

Tyr Xaa Asp Ala Ile Phe Thr Asn Ser Xaa Arg Lys Val Leu Gly Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30


<210> SEQ ID NO 39
<211> LENGTH: 33
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(33)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 39
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Tyr Xaa Asp Ala Ile Phe Thr Asn Ser Xaa Arg Lys Val Leu Gly Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa


<210> SEQ ID NO 40
<211> LENGTH: 34
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(34)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 40

Tyr Xaa Asp Ala Ile Phe Thr Asn Ser Xaa Arg Lys Val Leu Gly Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa


<210> SEQ ID NO 41
<211> LENGTH: 35
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(35)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 41

Tyr Xaa Asp Ala Ile Phe Thr Asn Ser Xaa Arg Lys Val Leu Gly Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa
        35


<210> SEQ ID NO 42
<211> LENGTH: 36
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
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<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(36)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 42

Tyr Xaa Asp Ala Ile Phe Thr Asn Ser Xaa Arg Lys Val Leu Gly Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa
        35


<210> SEQ ID NO 43
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(37)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 43

Tyr Xaa Asp Ala Ile Phe Thr Asn Ser Xaa Arg Lys Val Leu Gly Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa
        35


<210> SEQ ID NO 44
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(38)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 44
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Tyr Xaa Asp Ala Ile Phe Thr Asn Ser Xaa Arg Lys Val Leu Gly Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa
        35


<210> SEQ ID NO 45
<211> LENGTH: 39
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(39)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 45

Tyr Xaa Asp Ala Ile Phe Thr Asn Ser Xaa Arg Lys Val Leu Gly Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35


<210> SEQ ID NO 46
<211> LENGTH: 40
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(40)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 46

Tyr Xaa Asp Ala Ile Phe Thr Asn Ser Xaa Arg Lys Val Leu Gly Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35                  40


<210> SEQ ID NO 47
<211> LENGTH: 41
<212> TYPE: PRT
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(41)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 47

Tyr Xaa Asp Ala Ile Phe Thr Asn Ser Xaa Arg Lys Val Leu Gly Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35                  40


<210> SEQ ID NO 48
<211> LENGTH: 42
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(42)
<223> OTHER INFORMATION: Xaa is ay amino acid

<400> SEQUENCE: 48

Tyr Xaa Asp Ala Ile Phe Thr Asn Ser Xaa Arg Lys Val Leu Gly Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35                  40


<210> SEQ ID NO 49
<211> LENGTH: 43
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(43)
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<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 49

Tyr Xaa Asp Ala Ile Phe Thr Asn Ser Xaa Arg Lys Val Leu Gly Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35                  40


<210> SEQ ID NO 50
<211> LENGTH: 44
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(44)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 50

Tyr Xaa Asp Ala Ile Phe Thr Asn Ser Xaa Arg Lys Val Leu Gly Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35                  40


<210> SEQ ID NO 51
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is D-Ala

<400> SEQUENCE: 51

Tyr Xaa Asp Ala Ile Phe Thr Asn Ser Xaa Arg Lys Val Leu Xaa Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg
            20                  25


<210> SEQ ID NO 52
<211> LENGTH: 30
<212> TYPE: PRT
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(30)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 52

Tyr Xaa Asp Ala Ile Phe Thr Asn Ser Xaa Arg Lys Val Leu Xaa Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa
            20                  25                  30


<210> SEQ ID NO 53
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(31)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 53

Tyr Xaa Asp Ala Ile Phe Thr Asn Ser Xaa Arg Lys Val Leu Xaa Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa
            20                  25                  30


<210> SEQ ID NO 54
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is D-Tyr
<220> FEATURE:
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<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(32)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 54

Tyr Xaa Asp Ala Ile Phe Thr Asn Ser Xaa Arg Lys Val Leu Xaa Gln
1 5 10 15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
20 25 30

<210> SEQ ID NO 55
<211> LENGTH: 33
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(33)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 55

Tyr Xaa Asp Ala Ile Phe Thr Asn Ser Xaa Arg Lys Val Leu Xaa Gln
1 5 10 15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
20 25 30

Xaa

<210> SEQ ID NO 56
<211> LENGTH: 34
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(34)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 56

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Tyr Xaa Asp Ala Ile Phe Thr Asn Ser Xaa Arg Lys Val Leu Xaa Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa


<210> SEQ ID NO 57
<211> LENGTH: 35
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(35)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 57

Tyr Xaa Asp Ala Ile Phe Thr Asn Ser Xaa Arg Lys Val Leu Xaa Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa
        35


<210> SEQ ID NO 58
<211> LENGTH: 36
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(36)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 58

Tyr Xaa Asp Ala Ile Phe Thr Asn Ser Xaa Arg Lys Val Leu Xaa Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa
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        35


<210> SEQ ID NO 59
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(37)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 59

Tyr Xaa Asp Ala Ile Phe Thr Asn Ser Xaa Arg Lys Val Leu Xaa Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa
        35


<210> SEQ ID NO 60
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(38)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 60

Tyr Xaa Asp Ala Ile Phe Thr Asn Ser Xaa Arg Lys Val Leu Xaa Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa
        35


<210> SEQ ID NO 61
<211> LENGTH: 39
<212> TYPE: PRT
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(39)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 61

Tyr Xaa Asp Ala Ile Phe Thr Asn Ser Xaa Arg Lys Val Leu Xaa Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa
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<210> SEQ ID NO 62
<211> LENGTH: 40
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(40)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 62

Tyr Xaa Asp Ala Ile Phe Thr Asn Ser Xaa Arg Lys Val Leu Xaa Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35                  40


<210> SEQ ID NO 63
<211> LENGTH: 41
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
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<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(41)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 63

Tyr Xaa Asp Ala Ile Phe Thr Asn Ser Xaa Arg Lys Val Leu Xaa Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35                  40


<210> SEQ ID NO 64
<211> LENGTH: 42
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(42)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 64

Tyr Xaa Asp Ala Ile Phe Thr Asn Ser Xaa Arg Lys Val Leu Xaa Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35                  40


<210> SEQ ID NO 65
<211> LENGTH: 43
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is D-Tyr
<220> FEATURE:
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<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(43)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 65

Tyr Xaa Asp Ala Ile Phe Thr Asn Ser Xaa Arg Lys Val Leu Xaa Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35                  40


<210> SEQ ID NO 66
<211> LENGTH: 44
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is D-Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(44)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 66

Tyr Xaa Asp Ala Ile Phe Thr Asn Ser Xaa Arg Lys Val Leu Xaa Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35                  40


<210> SEQ ID NO 67
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 67

Tyr Ala Asp Ala Ile Phe Thr Ala Ser Tyr Arg Lys Val Leu Ala Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Ala Leu Gln Asp Ile Met Ser Arg
            20                  25


<210> SEQ ID NO 68
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 68

Tyr Ala Asp Ala Ile Phe Thr Ala Ala Tyr Arg Lys Val Leu Ala Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Ala Leu Gln Asp Ile Met Ser Arg
            20                  25


<210> SEQ ID NO 69
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 69

Tyr Ala Asp Ala Ile Phe Thr Asn Ser Tyr Arg Lys Val Leu Gly Gln
1               5                   10                  15

Leu Ser Ala Arg Lys Lys Leu Gln Asp Ile Met Ser Arg
            20                  25


<210> SEQ ID NO 70
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa is Asn, D-Asn or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa is Ser or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Tyr or D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is Gly, D-Ala or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (21)..(21)
<223> OTHER INFORMATION: Xaa is Lys or D-Lys
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Xaa is Leu, D-Leu, Lys or Ala

<400> SEQUENCE: 70

Tyr Xaa Asp Ala Ile Phe Thr Xaa Xaa Xaa Arg Lys Val Leu Xaa Gln
1               5                   10                  15

Leu Ser Ala Arg Xaa Xaa Leu Gln Asp Ile Met Ser Arg
            20                  25


<210> SEQ ID NO 71
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa is Asn, D-Asn or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa is Ser or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Tyr or D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is Gly, D-Ala or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (21)..(21)
<223> OTHER INFORMATION: Xaa is Lys or D-Lys
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Xaa is Leu, D-Leu, Lys or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(30)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 71

Tyr Xaa Asp Ala Ile Phe Thr Xaa Xaa Xaa Arg Lys Val Leu Xaa Gln
1               5                   10                  15

Leu Ser Ala Arg Xaa Xaa Leu Gln Asp Ile Met Ser Arg Xaa
            20                  25                  30


<210> SEQ ID NO 72
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa is Asn, D-Asn or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa is Ser or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Tyr or D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is Gly, D-Ala or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (21)..(21)
<223> OTHER INFORMATION: Xaa is D-Lys or Lys
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (22)..(22)
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<223> OTHER INFORMATION: Xaa is Leu, D-Leu, Lys or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(31)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 72

Tyr Xaa Asp Ala Ile Phe Thr Xaa Xaa Xaa Arg Lys Val Leu Xaa Gln
1               5                   10                  15

Leu Ser Ala Arg Xaa Xaa Leu Gln Asp Ile Met Ser Arg Xaa Xaa
            20                  25                  30


<210> SEQ ID NO 73
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa is Asn, D-Asn or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa is Ser or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Tyr or D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is Gly, D-Ala or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (21)..(21)
<223> OTHER INFORMATION: Xaa is Lys or D-Lys
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Xaa is Leu, D-Leu, Lys or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(32)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 73

Tyr Xaa Asp Ala Ile Phe Thr Xaa Xaa Xaa Arg Lys Val Leu Xaa Gln
1               5                   10                  15

Leu Ser Ala Arg Xaa Xaa Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30


<210> SEQ ID NO 74
<211> LENGTH: 33
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (8)..(8)
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<223> OTHER INFORMATION: Xaa is Asn, D-Asn or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa is Ser or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Tyr or D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is Gly, D-Ala or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (21)..(21)
<223> OTHER INFORMATION: Xaa is Lys or D-Lys
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Xaa is Leu, D-Leu, Lys or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(33)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 74

Tyr Xaa Asp Ala Ile Phe Thr Xaa Xaa Xaa Arg Lys Val Leu Xaa Gln
1               5                   10                  15

Leu Ser Ala Arg Xaa Xaa Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa


<210> SEQ ID NO 75
<211> LENGTH: 34
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa is Asn, D-Asn or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa is Ser or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Tyr or D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is Gly, D-Ala or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (21)..(21)
<223> OTHER INFORMATION: Xaa is Lys or D-Lys
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Xaa is Leu, D-Leu, Lys or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(34)
<223> OTHER INFORMATION: Xaa is any amino acid
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<400> SEQUENCE: 75

Tyr Xaa Asp Ala Ile Phe Thr Xaa Xaa Xaa Arg Lys Val Leu Xaa Gln
1               5                   10                  15

Leu Ser Ala Arg Xaa Xaa Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa


<210> SEQ ID NO 76
<211> LENGTH: 35
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa is Asn, D-Asn or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa is Ser or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Tyr or D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is Gly, D-Ala or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (21)..(21)
<223> OTHER INFORMATION: Xaa is Lys or D-Lys
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Xaa is Leu, D-Leu, Lys or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(35)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 76

Tyr Xaa Asp Ala Ile Phe Thr Xaa Xaa Xaa Arg Lys Val Leu Xaa Gln
1               5                   10                  15

Leu Ser Ala Arg Xaa Xaa Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa
        35


<210> SEQ ID NO 77
<211> LENGTH: 36
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa is Asn, D-Asn or Ala
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<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa is Ser or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Tyr or D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is Gly, D-Ala or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (21)..(21)
<223> OTHER INFORMATION: Xaa is Lys or D-Lys
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Xaa is Leu, D-Leu, Lys or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(36)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 77

Tyr Xaa Asp Ala Ile Phe Thr Xaa Xaa Xaa Arg Lys Val Leu Xaa Gln
1               5                   10                  15

Leu Ser Ala Arg Xaa Xaa Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa
        35


<210> SEQ ID NO 78
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa Asn, D-Asn or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa is Ser or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Tyr or D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is Gly, D-Ala or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (21)..(21)
<223> OTHER INFORMATION: Xaa is Lys or D-Lys
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Xaa is Leu, D-Leu, Lys or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(37)
<223> OTHER INFORMATION: Xaa is any amino acid
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<400> SEQUENCE: 78

Tyr Xaa Asp Ala Ile Phe Thr Xaa Xaa Xaa Arg Lys Val Leu Xaa Gln
1               5                   10                  15

Leu Ser Ala Arg Xaa Xaa Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa
        35


<210> SEQ ID NO 79
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa is Asn, D-Asn or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa is Ser or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Tyr or D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is Gly, D-Ala or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (21)..(21)
<223> OTHER INFORMATION: Xaa is Lys or D-Lys
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Xaa Leu, D-Leu, Lys or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(38)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 79

Tyr Xaa Asp Ala Ile Phe Thr Xaa Xaa Xaa Arg Lys Val Leu Xaa Gln
1               5                   10                  15

Leu Ser Ala Arg Xaa Xaa Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa
        35


<210> SEQ ID NO 80
<211> LENGTH: 39
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (8)..(8)
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<223> OTHER INFORMATION: Xaa is Asn, D-Asn or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa is Ser or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Tyr or D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is Gly, D-Ala or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (21)..(21)
<223> OTHER INFORMATION: Xaa is Lys or D-Lys
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Xaa is Leu, D-Leu, Lys or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(39)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 80

Tyr Xaa Asp Ala Ile Phe Thr Xaa Xaa Xaa Arg Lys Val Leu Xaa Gln
1               5                   10                  15

Leu Ser Ala Arg Xaa Xaa Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35


<210> SEQ ID NO 81
<211> LENGTH: 40
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa is Asn, D-Asn or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa is Ser or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Tyr or D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is Gly, D-Ala or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (21)..(21)
<223> OTHER INFORMATION: Xaa is Lys or D-Lys
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Xaa is Leu, D-Leu, Lys or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(40)
<223> OTHER INFORMATION: Xaa is any amino acid
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<400> SEQUENCE: 81

Tyr Xaa Asp Ala Ile Phe Thr Xaa Xaa Xaa Arg Lys Val Leu Xaa Gln
1               5                   10                  15

Leu Ser Ala Arg Xaa Xaa Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35                  40


<210> SEQ ID NO 82
<211> LENGTH: 41
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa is Asn, D-Asn or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa is Ser or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Tyr or D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is Gly, D-Ala or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (21)..(21)
<223> OTHER INFORMATION: Xaa is Lys or D-Lys
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Xaa is Leu, D-Leu, Lys or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(41)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 82

Tyr Xaa Asp Ala Ile Phe Thr Xaa Xaa Xaa Arg Lys Val Leu Xaa Gln
1               5                   10                  15

Leu Ser Ala Arg Xaa Xaa Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35                  40


<210> SEQ ID NO 83
<211> LENGTH: 42
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
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<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa is Asn, D-Asn or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa is Ser or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Tyr or D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is Gly, D-Ala or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (21)..(21)
<223> OTHER INFORMATION: Xaa is Lys or D-Lys
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Xaa is Leu, D-Leu, Lys or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(42)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 83

Tyr Xaa Asp Ala Ile Phe Thr Xaa Xaa Xaa Arg Lys Val Leu Xaa Gln
1               5                   10                  15

Leu Ser Ala Arg Xaa Xaa Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35                  40


<210> SEQ ID NO 84
<211> LENGTH: 43
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa is Asn, D-Asn or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa is Ser or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Tyr or D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is Gly, D-Ala or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (21)..(21)
<223> OTHER INFORMATION: Xaa is Lys or D-Lys
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Xaa is Leu, D-Leu, Lys or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(43)
```

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<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 84

Tyr Xaa Asp Ala Ile Phe Thr Xaa Xaa Xaa Arg Lys Val Leu Xaa Gln
1               5                   10                  15

Leu Ser Ala Arg Xaa Xaa Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35                  40


<210> SEQ ID NO 85
<211> LENGTH: 44
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is D-Ala or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa is Asn, D-Asn or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa is Ser or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa is Tyr or D-Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is Gly, D-Ala or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (21)..(21)
<223> OTHER INFORMATION: Xaa is Lys or D-Lys
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Xaa is Leu, D-Leu, Lys or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(44)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 85

Tyr Xaa Asp Ala Ile Phe Thr Xaa Xaa Xaa Arg Lys Val Leu Xaa Gln
1               5                   10                  15

Leu Ser Ala Arg Xaa Xaa Leu Gln Asp Ile Met Ser Arg Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35                  40


<210> SEQ ID NO 86
<211> LENGTH: 2032
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 86

attgcacagc tgccagtggc ctcctgagca gcgaggggca gcgcatctgc ctccaggaat     60
```

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gtagggctca gagacacggg accagaaatc ctcagggagg ctgtggcttt gcttccagaa    120

gatcctgaga gctccagtgt ccacttcttg ccatccttct gtatgagtgg gaaaccaacc    180

caggccaccc ttgggggcag gtcctgggac ttcttggagc agatgagcct ttatccatgc    240

gacagaacag gaggtaccta ctgacaccca tgtcctagga tagacacatc tgtatgtggg    300

tgcgaggcac tgggtcacct ccacctagaa tgtgacttca tcactcagct gagagacgat    360

gagcttgcat gccttcaggc ggcagagggg accaacaact cgtccatggg atgccctggg    420

acctgggatg ggctgctgtg ctggccccca actggctctg gccagtgggt ctccctcccc    480

tgccctgaat tcttctctca ttttggctca gacccagggg ctgtgaaaag ggactgcacc    540

atcacgggtt ggtctgatcc cttcccacca tatcccgtgg cctgtcctgt gcccttggaa    600

ctgctaacag aggagaagtc ttacttctcc acggtgaaga tcatctacac cacaggccac    660

agcatctcca ttgtagccct ctgcgtggct attgccatcc tggttgctct caggaggctc    720

cactgcccca ggaactacat ccacacgcag ctgtttgcta ctttcatcct caaggccagt    780

gctgtgttcc tgaaggatgc tgctgtcttc cagggtgata gcacggacca ctgcagcatg    840

tccactattc tgtgcaaggt ctctgtggcc gtctcacatt ttgccaccat gaccaacttc    900

agctggctgc tggcagaagc cgtctacctg agctgtctgt tggcctccac atctcctagg    960

tccaaaccag ctttctggtg gctggttctc gctggctggg gactccctgt gctatgcact   1020

ggtacgtggg tgggctgcaa actggctttt gaggacactg cgtgctggga cctagacgac   1080

agctccccct actggtggat catcaaaggg cccatagtcc tctctgttgg ggtgaacttt   1140

gggctatttc tcaatataat ttgcatcctg ctgaggaagc tggggcctgc acaaggcggc   1200

ttacacacac gggctcagta ctggcggctt tccaaatcaa cacttctcct tatcccgctg   1260

tttggaattc attacatcat cttcaacttc ctgcctgaca gtgctggcct tggcatccgt   1320

ctacccctgg agctgggact ggggtccttc cagggttttg ttgttgctgt cctctactgc   1380

ttcctcaatc aagaggtgag gacggagatt tcacgcaaat ggtatggcca tgaccctgaa   1440

cttctgccag ctcggcggac ctgcactgag tggaccacac ctccccgatc gagagtgaag   1500

gtgctcacct ctgagtgcta ggccagccat cacaaaggcc gagccccaaa accctgcact   1560

caaactgcca tgccaccaag ggcaacaagg tcctcccttc cgttctcgtt ctctgcatct   1620

gctttctcta ggtccctgta taccaacctc cgactttctc agttcctgta tgcccccatc   1680

tgttctttct tcctatctag ggctattgcc caaggcccag ggaaaccaat aaacttgtac   1740

atgagtgatc tgcagttgag tcaatgtggc tctgaagggg agctcttgtc agcagccatt   1800

atttgcactt ccggtgcatt cctcatccct tggctgcagc tgcctcattg ccatttgtct   1860

ctatatgaat tctcatgttt tatttatttt ttaaaagcac tcttttgtct caaccccccac   1920

tccccttccc ctcctcccgc aggcaaaatg tgaatctttg cttctctgtc ttgcaagtgt   1980

ggagtgtacc acatgtctgt atttggatgt aataaatgct atctgttatg tg           2032
```

<210> SEQ ID NO 87
<211> LENGTH: 1116
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 87

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atgggatgcc ctgggacctg ggatgggctg ctgtgctggc cccaactgg ctctggccag      60

tgggtctccc tcccctgccc tgaattcttc tctcattttg gctcagaccc aggggctgtg    120

aaaagggact gcaccatcac gggttggtct gatcccttcc caccatatcc cgtggcctgt    180

cctgtgccct tggaactgct aacagaggag aagtcttact tctccacggt gaagatcatc    240

tacaccacag gccacagcat ctccattgta gccctctgcg tggctattgc catcctggtt    300

gctctcagga ggctccactg ccccaggaac tacatccaca cgcagctgtt tgctactttc    360

atcctcaagg ccagtgctgt gttcctgaag gatgctgctg tcttccaggg tgatagcacg    420

gaccactgca gcatgtccac tattctgtgc aaggtctctg tggccgtctc acattttgcc    480

accatgacca acttcagctg gctgctggca gaagccgtct acctgagctg tctgttggcc    540

tccacatctc ctaggtccaa accagctttc tggtggctgg ttctcgctgg ctggggactc    600

cctgtgctat gcactggtac gtgggtgggc tgcaaactgg cttttgagga cactgcgtgc    660

tgggacctag acgacagctc cccctactgg tggatcatca aagggcccat agtcctctct    720

gttggggtga actttgggct atttctcaat ataatttgca tcctgctgag gaagctgggg    780

cctgcacaag gcggcttaca cacacgggct cagtactggc ggctttccaa atcaacactt    840

ctccttatcc cgctgtttgg aattcattac atcatcttca acttcctgcc tgacagtgct    900

ggccttggca tccgtctacc cctggagctg ggactggggt ccttccaggg ttttgttgtt    960

gctgtcctct actgcttcct caatcaagag gtgaggacgg agatttcacg caaatggtat   1020

ggccatgacc ctgaacttct gccagctcgg cggacctgca ctgagtggac cacacctccc   1080

cgatcgagag tgaaggtgct cacctctgag tgctag                             1116
```

```
<210> SEQ ID NO 88
<211> LENGTH: 371
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 88

Met Gly Cys Pro Gly Thr Trp Asp Gly Leu Leu Cys Trp Pro Pro Thr
1               5                   10                  15

Gly Ser Gly Gln Trp Val Ser Leu Pro Cys Pro Glu Phe Phe Ser His
            20                  25                  30

Phe Gly Ser Asp Pro Gly Ala Val Lys Arg Asp Cys Thr Ile Thr Gly
        35                  40                  45

Trp Ser Asp Pro Phe Pro Pro Tyr Pro Val Ala Cys Pro Val Pro Leu
    50                  55                  60

Glu Leu Leu Thr Glu Glu Lys Ser Tyr Phe Ser Thr Val Lys Ile Ile
65                  70                  75                  80

Tyr Thr Thr Gly His Ser Ile Ser Ile Val Ala Leu Cys Val Ala Ile
                85                  90                  95

Ala Ile Leu Val Ala Leu Arg Arg Leu His Cys Pro Arg Asn Tyr Ile
            100                 105                 110

His Thr Gln Leu Phe Ala Thr Phe Ile Leu Lys Ala Ser Ala Val Phe
        115                 120                 125

Leu Lys Asp Ala Ala Val Phe Gln Gly Asp Ser Thr Asp His Cys Ser
    130                 135                 140

Met Ser Thr Ile Leu Cys Lys Val Ser Val Ala Val Ser His Phe Ala
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145                 150                 155                 160

Thr Met Thr Asn Phe Ser Trp Leu Leu Ala Glu Ala Val Tyr Leu Ser
                165                 170                 175

Cys Leu Leu Ala Ser Thr Ser Pro Arg Ser Lys Pro Ala Phe Trp Trp
            180                 185                 190

Leu Val Leu Ala Gly Trp Gly Leu Pro Val Leu Cys Thr Gly Thr Trp
        195                 200                 205

Val Gly Cys Lys Leu Ala Phe Glu Asp Thr Ala Cys Trp Asp Leu Asp
    210                 215                 220

Asp Ser Ser Pro Tyr Trp Trp Ile Ile Lys Gly Pro Ile Val Leu Ser
225                 230                 235                 240

Val Gly Val Asn Phe Gly Leu Phe Leu Asn Ile Ile Cys Ile Leu Leu
                245                 250                 255

Arg Lys Leu Gly Pro Ala Gln Gly Gly Leu His Thr Arg Ala Gln Tyr
            260                 265                 270

Trp Arg Leu Ser Lys Ser Thr Leu Leu Leu Ile Pro Leu Phe Gly Ile
        275                 280                 285

His Tyr Ile Ile Phe Asn Phe Leu Pro Asp Ser Ala Gly Leu Gly Ile
    290                 295                 300

Arg Leu Pro Leu Glu Leu Gly Leu Gly Ser Phe Gln Gly Phe Val Val
305                 310                 315                 320

Ala Val Leu Tyr Cys Phe Leu Asn Gln Glu Val Arg Thr Glu Ile Ser
                325                 330                 335

Arg Lys Trp Tyr Gly His Asp Pro Glu Leu Leu Pro Ala Arg Arg Thr
            340                 345                 350

Cys Thr Glu Trp Thr Thr Pro Pro Arg Ser Arg Val Lys Val Leu Thr
        355                 360                 365

Ser Glu Cys
    370
```

<210> SEQ ID NO 89
<211> LENGTH: 52
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 89

```
Met Asp Ser Leu Leu Trp Ala Thr Trp Val Leu Cys Leu Leu Asn Leu
1               5                   10                  15

Trp Gly Val Ala Leu Gly His Leu His Leu Glu Cys Asp Phe Ile Thr
            20                  25                  30

Gln Leu Arg Asp Asp Glu Leu Ala Cys Leu Gln Ala Ala Glu Gly Thr
        35                  40                  45

Asn Asn Ser Ser
    50
```

<210> SEQ ID NO 90
<211> LENGTH: 79
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 90

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```
Met Gln Leu Gly Gly Ile Ala Arg Asp Ser Leu Gly Gly Lys Gly His
1               5                   10                  15

Gly Ser Leu Gly Arg Thr Thr Gly Thr Thr Ala Met Asp Ser Leu Leu
            20                  25                  30

Trp Ala Thr Trp Val Leu Cys Leu Leu Asn Leu Trp Gly Val Ala Leu
        35                  40                  45

Gly His Leu His Leu Glu Cys Asp Phe Ile Thr Gln Leu Arg Asp Asp
    50                  55                  60

Glu Leu Ala Cys Leu Gln Ala Ala Glu Gly Thr Asn Asn Ser Ser
65                  70                  75


<210> SEQ ID NO 91
<211> LENGTH: 400
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 91

Met His Pro Glu Cys Asp Phe Ile Thr Gln Leu Arg Glu Asp Glu Ser
1               5                   10                  15

Ala Cys Leu Gln Ala Ala Glu Glu Met Pro Asn Thr Thr Leu Gly Cys
            20                  25                  30

Pro Ala Thr Trp Asp Gly Leu Leu Cys Trp Pro Thr Ala Gly Ser Gly
        35                  40                  45

Glu Trp Val Thr Leu Pro Cys Pro Asp Phe Phe Ser His Phe Ser Ser
    50                  55                  60

Glu Ser Gly Ala Val Lys Arg Asp Cys Thr Ile Thr Gly Trp Ser Glu
65                  70                  75                  80

Pro Phe Pro Pro Tyr Pro Val Ala Cys Pro Val Pro Leu Glu Leu Leu
                85                  90                  95

Ala Glu Glu Glu Ser Tyr Phe Ser Thr Val Lys Ile Ile Tyr Thr Val
            100                 105                 110

Gly His Ser Ile Ser Ile Val Ala Leu Phe Val Ala Ile Thr Ile Leu
        115                 120                 125

Val Ala Leu Arg Arg Leu His Cys Pro Arg Asn Tyr Val His Thr Gln
    130                 135                 140

Leu Phe Thr Thr Phe Ile Leu Lys Ala Gly Ala Val Phe Leu Lys Asp
145                 150                 155                 160

Ala Ala Leu Phe His Ser Asp Asp Thr Asp His Cys Ser Phe Ser Thr
                165                 170                 175

Val Leu Cys Lys Val Ser Val Ala Ala Ser His Phe Ala Thr Met Thr
            180                 185                 190

Asn Phe Ser Trp Leu Leu Ala Glu Ala Val Tyr Leu Asn Cys Leu Leu
        195                 200                 205

Ala Ser Thr Ser Pro Ser Ser Arg Arg Ala Phe Trp Trp Leu Val Leu
    210                 215                 220

Ala Gly Trp Gly Leu Pro Val Leu Phe Thr Gly Thr Trp Val Ser Cys
225                 230                 235                 240

Lys Leu Ala Phe Glu Asp Ile Ala Cys Trp Asp Leu Asp Asp Thr Ser
                245                 250                 255

Pro Tyr Trp Trp Ile Ile Lys Gly Pro Ile Val Leu Ser Val Gly Val
            260                 265                 270
```

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```
Asn Phe Gly Leu Phe Leu Asn Ile Ile Arg Ile Leu Val Arg Lys Leu
        275                 280                 285

Glu Pro Ala Gln Gly Ser Leu His Thr Gln Ser Gln Tyr Trp Arg Leu
    290                 295                 300

Ser Lys Ser Thr Leu Phe Leu Ile Pro Leu Phe Gly Ile His Tyr Ile
305                 310                 315                 320

Ile Phe Asn Phe Leu Pro Asp Asn Ala Gly Leu Gly Ile Arg Leu Pro
                325                 330                 335

Leu Glu Leu Gly Leu Gly Ser Phe Gln Gly Phe Ile Val Ala Ile Leu
            340                 345                 350

Tyr Cys Phe Leu Asn Gln Glu Val Arg Thr Glu Ile Ser Arg Lys Trp
        355                 360                 365

His Gly His Asp Pro Glu Leu Leu Pro Ala Trp Arg Thr Arg Ala Lys
    370                 375                 380

Trp Thr Thr Pro Ser Arg Ser Ala Ala Lys Val Leu Thr Ser Met Cys
385                 390                 395                 400


<210> SEQ ID NO 92
<211> LENGTH: 416
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 92

Met Trp Thr Thr Glu Pro Arg Lys Thr Pro Lys Trp Leu Gly Ser Ser
1               5                   10                  15

Cys Ser Leu Phe Pro Ala Ala Ser Pro Leu Gly Leu Leu Gly Gln Pro
            20                  25                  30

Cys Thr Trp Ala Glu Ser Leu Leu Leu Leu Ala Leu Tyr Pro Gly Cys
        35                  40                  45

Pro Ala Thr Trp Asp Gly Leu Leu Cys Trp Pro Thr Ala Gly Ser Gly
    50                  55                  60

Glu Trp Val Thr Leu Pro Cys Pro Asp Phe Phe Ser His Phe Ser Ser
65                  70                  75                  80

Glu Ser Gly Ala Val Lys Arg Asp Cys Thr Ile Thr Gly Trp Ser Glu
                85                  90                  95

Pro Phe Pro Pro Tyr Pro Val Ala Cys Pro Val Pro Leu Glu Leu Leu
            100                 105                 110

Ala Glu Glu Glu Ser Tyr Phe Ser Thr Val Lys Ile Ile Tyr Thr Val
        115                 120                 125

Gly His Ser Ile Ser Ile Val Ala Leu Phe Val Ala Ile Thr Ile Leu
    130                 135                 140

Val Ala Leu Arg Arg Leu His Cys Pro Arg Asn Tyr Val His Thr Gln
145                 150                 155                 160

Leu Phe Thr Thr Phe Ile Leu Lys Ala Gly Ala Val Phe Leu Lys Asp
                165                 170                 175

Ala Ala Leu Phe His Ser Asp Asp Thr Asp His Cys Ser Phe Ser Thr
            180                 185                 190

Val Leu Cys Lys Val Ser Val Ala Ala Ser His Phe Ala Thr Met Thr
        195                 200                 205

Asn Phe Ser Trp Leu Leu Ala Glu Ala Val Tyr Leu Asn Cys Leu Leu
    210                 215                 220
```

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Ala Ser Thr Ser Pro Ser Ser Arg Arg Ala Phe Trp Trp Leu Val Leu
225                 230                 235                 240

Ala Gly Trp Gly Leu Pro Val Leu Phe Thr Gly Thr Trp Val Ser Cys
                245                 250                 255

Lys Leu Ala Phe Glu Asp Ile Ala Cys Trp Asp Leu Asp Asp Thr Ser
            260                 265                 270

Pro Tyr Trp Trp Ile Ile Lys Gly Pro Ile Val Leu Ser Val Gly Val
        275                 280                 285

Asn Phe Gly Leu Phe Leu Asn Ile Ile Arg Ile Leu Val Arg Lys Leu
    290                 295                 300

Glu Pro Ala Gln Gly Ser Leu His Thr Gln Ser Gln Tyr Trp Arg Leu
305                 310                 315                 320

Ser Lys Ser Thr Leu Phe Leu Ile Pro Leu Phe Gly Ile His Tyr Ile
                325                 330                 335

Ile Phe Asn Phe Leu Pro Asp Asn Ala Gly Leu Gly Ile Arg Leu Pro
            340                 345                 350

Leu Glu Leu Gly Leu Gly Ser Phe Gln Gly Phe Ile Val Ala Ile Leu
        355                 360                 365

Tyr Cys Phe Leu Asn Gln Glu Val Arg Thr Glu Ile Ser Arg Lys Trp
    370                 375                 380

His Gly His Asp Pro Glu Leu Leu Pro Ala Trp Arg Thr Arg Ala Lys
385                 390                 395                 400

Trp Thr Thr Pro Ser Arg Ser Ala Ala Lys Val Leu Thr Ser Met Cys
                405                 410                 415
```

<210> SEQ ID NO 93
<211> LENGTH: 1537
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 93

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aaacacacct gaaactactg tgagcaggta ttgggccaca tgcacccaga atgtgacttc     60

atcacccagc tgagagagga tgagagtgcc tgtctacaag cagcagagga gatgcccaac    120

accaccctgg gctgccctgc gacctgggat gggctgctgt gctggccaac ggcaggctct    180

ggcgagtggg tcaccctccc ctgcccggat ttcttctctc acttcagctc agagtcaggg    240

gctgtgaaac gggattgtac tatcactggc tggtctgagc cctttccacc ttaccctgtg    300

gcctgccctg tgcctctgga gctgctggct gaggaggaat cttacttctc cacagtgaag    360

attatctaca ccgtgggcca tagcatctct attgtagccc tcttcgtggc catcaccatc    420

ctggttgctc tcaggaggct ccactgcccc cggaactacg tccacaccca gctgttcacc    480

acttttatcc tcaaggcggg agctgtgttc ctgaaggatg ctgccctttt ccacagcgac    540

gacactgacc actgcagctt ctccactgtt ctatgcaagg tctctgtggc cgcctcccat    600

ttcgccacca tgaccaactt cagctggctg ttggcagaag ccgtctacct gaactgcctc    660

ctggcctcca cctcccccag ctcaaggaga gccttctggt ggctggttct cgctggctgg    720

gggctgcccg tgctcttcac tggcacgtgg gtgagctgca aactggcctt cgaggacatc    780

gcgtgctggg acctggacga cacctccccc tactggtgga tcatcaaagg gcccattgtc    840

ctctcggtcg gggtgaactt tgggcttttt ctcaatatta tccgcatcct ggtgaggaaa    900
```

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ctggagccag ctcagggcag cctccatacc cagtctcagt attggcgtct ctccaagtcg 960

acacttttcc tgatcccact ctttggaatt cactacatca tcttcaactt cctgccagac 1020

aatgctggcc tgggcatccg cctccccctg gagctgggac tgggttcctt ccagggcttc 1080

attgttgcca tcctctactg cttcctcaac caagaggtga ggactgagat ctcacggaag 1140

tggcatggcc atgaccctga gcttctgcca gcctggagga cccgtgctaa gtggaccacg 1200

ccttcccgct cggcggcaaa ggtgctgaca tctatgtgct aggctgcctc atcacgccac 1260

tggagtccac acttgaattt gggcagctac cacgggtctg ccatgctctg gaggagcaag 1320

ggggccacat ccccacccca gctgttaccc agcccggggc aggtgcagcc cttcctccct 1380

gtctctgcct ctgactctct tttgaggtcc ctgtatgtct acctctgact tctgtggtcc 1440

ctctgtgtct gctctcatcc attcctctta ctggggcatg ggctctagc ccaaggctca 1500

gaggagccaa taaacctgta aatgaaaaaa aaaaaaa 1537

<210> SEQ ID NO 94
<211> LENGTH: 1203
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 94

atgcacccag aatgtgactt catcacccag ctgagagagg atgagagtgc ctgtctacaa 60

gcagcagagg agatgcccaa caccaccctg ggctgccctg cgacctggga tgggctgctg 120

tgctggccaa cggcaggctc tggcgagtgg gtcaccctcc cctgcccgga tttcttctct 180

cacttcagct cagagtcagg ggctgtgaaa cgggattgta ctatcactgg ctggtctgag 240

ccctttccac cttaccctgt ggcctgccct gtgcctctgg agctgctggc tgaggaggaa 300

tcttacttct ccacagtgaa gattatctac accgtgggcc atagcatctc tattgtagcc 360

ctcttcgtgg ccatcaccat cctggttgct ctcaggaggc tccactgccc ccggaactac 420

gtccacaccc agctgttcac cacttttatc ctcaaggcgg gagctgtgtt cctgaaggat 480

gctgcccttt tccacagcga cgacactgac cactgcagct tctccactgt tctatgcaag 540

gtctctgtgg ccgcctccca tttcgccacc atgaccaact tcagctggct gttggcagaa 600

gccgtctacc tgaactgcct cctggcctcc acctccccca gctcaaggag agccttctgg 660

tggctggttc tcgctggctg gggggctgccc gtgctcttca ctggcacgtg ggtgagctgc 720

aaactggcct tcgaggacat cgcgtgctgg gacctggacg acacctcccc ctactggtgg 780

atcatcaaag ggcccattgt cctctcggtc ggggtgaact ttgggctttt tctcaatatt 840

atccgcatcc tggtgaggaa actggagcca gctcagggca gcctccatac ccagtctcag 900

tattggcgtc tctccaagtc gacacttttc ctgatcccac tctttggaat tcactacatc 960

atcttcaact tcctgccaga caatgctggc ctgggcatcc gcctccccct ggagctggga 1020

ctgggttcct tccagggctt cattgttgcc atcctctact gcttcctcaa ccaagaggtg 1080

aggactgaga tctcacggaa gtggcatggc catgaccctg agcttctgcc agcctggagg 1140

acccgtgcta agtggaccac gccttcccgc tcggcggcaa aggtgctgac atctatgtgc 1200

tag 1203

<210> SEQ ID NO 95

<211> LENGTH: 1671
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 95

cttccttcct tccttccttc cttccatctg aacatctacc aaacacacct gaaactactg 60

tgagcaggtc tttgcctagg attagggcac agatatgaat caggccttgt ccctgttctc 120

aggtcatgtg gaccacagag cccagaaaga cacccaaatg gcttggctca tcctgttcac 180

tgtttccagc agcctcaccc ctcggattat tgggacagcc ctgcacctgg gctgagtctc 240

tgctgctcct ggctctctat ccaggctgcc ctgcgacctg ggatgggctg ctgtgctggc 300

caacggcagg ctctggcgag tgggtcaccc tcccctgccc ggatttcttc tctcacttca 360

gctcagagtc aggggctgtg aaacgggatt gtactatcac tggctggtct gagccctttc 420

caccttaccc tgtggcctgc cctgtgcctc tggagctgct ggctgaggag gaatcttact 480

tctccacagt gaagattatc tacaccgtgg gccatagcat ctctattgta gccctcttcg 540

tggccatcac catcctggtt gctctcagga ggctccactg cccccggaac tacgtccaca 600

cccagctgtt caccactttt atcctcaagg cgggagctgt gttcctgaag gatgctgccc 660

ttttccacag cgacgacact gaccactgca gcttctccac tgttctatgc aaggtctctg 720

tggccgcctc ccatttcgcc accatgacca acttcagctg gctgttggca gaagccgtct 780

acctgaactg cctcctggcc tccacctccc ccagctcaag gagagccttc tggtggctgg 840

ttctcgctgg ctgggggctg cccgtgctct tcactggcac gtgggtgagc tgcaaactgg 900

ccttcgagga catcgcgtgc tgggacctgg acgacacctc cccctactgg tggatcatca 960

aagggcccat tgtcctctcg gtcggggtga actttgggct tttctcaat attatccgca 1020

tcctggtgag gaaactggag ccagctcagg gcagcctcca tacccagtct cagtattggc 1080

gtctctccaa gtcgacactt ttcctgatcc cactctttgg aattcactac atcatcttca 1140

acttcctgcc agacaatgct ggcctgggca tccgcctccc cctggagctg ggactgggtt 1200

ccttccaggg cttcattgtt gccatcctct actgcttcct caaccaagag gtgaggactg 1260

agatctcacg gaagtggcat ggccatgacc ctgagcttct gccagcctgg aggacccgtg 1320

ctaagtggac cacgccttcc cgctcggcgg caaaggtgct gacatctatg tgctaggctg 1380

cctcatcacg ccactggagt ccacacttga atttgggcag ctaccacggg tctgccatgc 1440

tctggaggag caagggggcc acatccccac cccagctgtt acccagcccg gggcaggtgc 1500

agcccttcct ccctgtctct gcctctgact ctcttttgag gtccctgtat gtctacctct 1560

gacttctgtg gtccctctgt gtctgctctc atccattcct cttactgggg catggggctc 1620

tagcccaagg ctcagaggag ccaataaacc tgtaaatgaa aaaaaaaaaa a 1671

<210> SEQ ID NO 96
<211> LENGTH: 1251
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 96

atgtggacca cagagcccag aaagacaccc aaatggcttg gctcatcctg ttcactgttt 60

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ccagcagcct cacccctcgg attattggga cagccctgca cctgggctga gtctctgctg    120

ctcctggctc tctatccagg ctgccctgcg acctgggatg ggctgctgtg ctggccaacg    180

gcaggctctg gcgagtgggt caccctcccc tgcccggatt tcttctctca cttcagctca    240

gagtcagggg ctgtgaaacg ggattgtact atcactggct ggtctgagcc ctttccacct    300

taccctgtgg cctgccctgt gcctctggag ctgctggctg aggaggaatc ttacttctcc    360

acagtgaaga ttatctacac cgtgggccat agcatctcta ttgtagccct cttcgtggcc    420

atcaccatcc tggttgctct caggaggctc cactgccccc ggaactacgt ccacacccag    480

ctgttcacca cttttatcct caaggcggga gctgtgttcc tgaaggatgc tgcccttttc    540

cacagcgacg acactgacca ctgcagcttc tccactgttc tatgcaaggt ctctgtggcc    600

gcctcccatt tcgccaccat gaccaacttc agctggctgt tggcagaagc cgtctacctg    660

aactgcctcc tggcctccac ctcccccagc tcaaggagag ccttctggtg gctggttctc    720

gctggctggg ggctgcccgt gctcttcact ggcacgtggg tgagctgcaa actggccttc    780

gaggacatcg cgtgctggga cctggacgac acctccccct actggtggat catcaaaggg    840

cccattgtcc tctcggtcgg ggtgaacttt gggctttttc tcaatattat ccgcatcctg    900

gtgaggaaac tggagccagc tcagggcagc ctccataccc agtctcagta ttggcgtctc    960

tccaagtcga cacttttcct gatcccactc tttggaattc actacatcat cttcaacttc   1020

ctgccagaca atgctggcct gggcatccgc ctcccccctgg agctgggact gggttccttc   1080

cagggcttca ttgttgccat cctctactgc ttcctcaacc aagaggtgag gactgagatc   1140

tcacggaagt ggcatggcca tgaccctgag cttctgccag cctggaggac ccgtgctaag   1200

tggaccacgc cttcccgctc ggcggcaaag gtgctgacat ctatgtgcta g            1251


<210> SEQ ID NO 97
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 97

Met Asp Arg Arg Met Trp Gly Ala His Val Phe Cys Val Leu Ser Pro
1               5                   10                  15

Leu Pro Thr Val Leu Gly His
            20


<210> SEQ ID NO 98
<211> LENGTH: 53
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 98

Met Asp Arg Arg Met Trp Gly Ala His Val Phe Cys Val Leu Ser Pro
1               5                   10                  15

Leu Pro Thr Val Leu Gly His Met His Pro Glu Cys Asp Phe Ile Thr
            20                  25                  30

Gln Leu Arg Glu Asp Glu Ser Ala Cys Leu Gln Ala Ala Glu Glu Met
        35                  40                  45

Pro Asn Thr Thr Leu
```

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50

<210> SEQ ID NO 99
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 99

gggtgtgaac cacgagaaat 20

<210> SEQ ID NO 100
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 100

actgtggtca tgagcccttc 20

<210> SEQ ID NO 101
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 101

atgccactct gggtgttctt t 21

<210> SEQ ID NO 102
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 102

gcagtttgcg ggcatataat 20

<210> SEQ ID NO 103
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 103

ctctgcttgc tgaacctgtg 20

<210> SEQ ID NO 104
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 104

catcccatgg acgagttgtt 20

<210> SEQ ID NO 105

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<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 105

ctgctgtctt ccagggtgat                                                 20


<210> SEQ ID NO 106
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 106

taggagatgt ggaggccaac                                                 20


<210> SEQ ID NO 107
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 107

acttcctgcc tgacagtgct                                                 20


<210> SEQ ID NO 108
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 108

tggcagaagt tcagggtcat                                                 20


<210> SEQ ID NO 109
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 109

ctgctgagct ccctaccagt                                                 20


<210> SEQ ID NO 110
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 110

cagcccgagg aggagttg                                                   18


<210> SEQ ID NO 111
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 111

gcttctccac ggttctgtgc a                                              21


<210> SEQ ID NO 112
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 112

tgggtgacgt agaggccaag                                                20


<210> SEQ ID NO 113
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 113

gctccttcca gggcttcatt gt                                             22


<210> SEQ ID NO 114
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 114

gaaggctttg cccatttggc a                                              21


<210> SEQ ID NO 115
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 115

aagcagtggt atcaacgcag agtacgcggg                                     30


<210> SEQ ID NO 116
<211> LENGTH: 57
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (27)..(27)
<223> OTHER INFORMATION: a, g or c
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (28)..(28)
<223> OTHER INFORMATION: a, g, t or c
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (56)..(57)
<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 116
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aagcagtggt atcaacgcag agtacttttt tttttttttt tttttttttt tttttnn 57

<210> SEQ ID NO 117
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 117

ctaatacgac tcactatagg gcaagcagtg gtatcaacgc agagt 45

<210> SEQ ID NO 118
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 118

ctaatacgac tcactatagg gc 22

<210> SEQ ID NO 119
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 119

aagcagtggt atcaacgcag agt 23

<210> SEQ ID NO 120
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 120

cagcccaaag ttcaccccaa cagagagg 28

<210> SEQ ID NO 121
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 121

ccagtagggg gagctgtcgt ctaggtc 27

<210> SEQ ID NO 122
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 122

ctggtggctg gttctcgctg gctgg 25

<210> SEQ ID NO 123

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<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 123

tctcgctggc tggggactcc ctgtg                                           25


<210> SEQ ID NO 124
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 124

cctgtgctat gcactggta                                                  19


<210> SEQ ID NO 125
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 125

agacacggga ccagaaatc                                                  19


<210> SEQ ID NO 126
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 126

agcctttatc catgcgacag                                                 20


<210> SEQ ID NO 127
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 127

gatgcaaatt atattgagaa acag                                            24


<210> SEQ ID NO 128
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 128

ggatgctgcc cttttccaca gcgacgac                                        28


<210> SEQ ID NO 129
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 129

gggaggtgga ggccaggagg cag                                             23


<210> SEQ ID NO 130
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 130

cgacactgac cactgcagct tctccact                                        28


<210> SEQ ID NO 131
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 131

aggccaggag gcagttcagg tagacgg                                         27


<210> SEQ ID NO 132
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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1. A method for protecting or treating a mammal against oxidative renal damage, the method comprising the step of administering an effective amount of a ligand to GHRH renal receptor to the mammal.

2. The method of claim **1**, wherein the ligand is GHRH, a biologically active fragment of GHRH or a GHRH agonist thereof.

3. The method of claim **2**, wherein the ligand is selected from the group consisting of SEQ ID NO.:1, SEQ ID NO.:2 and SEQ ID NO.:3.

4. The method of claim **2**, wherein the ligand is selected from the group consisting of SEQ ID NO.:4, SEQ ID NO.:5, SEQ ID NO.:6, SEQ ID NO.:7, SEQ ID NO.:8, SEQ ID NO.:9 and SEQ ID NO.:10.

5. The method of claim **1** wherein the effective amount is not substantially active against anterior pituitary GHRH receptor.

6. The method of claim **5** wherein the effective amount has a protective effect substantially similar to a subcutaneous 1.0 mg rat GHRH(1-29)NH_2 dose per kilogram of body weight per day or lower, in a Sprague Dawley rat submitted to a high-salt diet.

7. The method of claim **6** wherein the effective amount has a protective effect substantially similar to subcutaneous 0.5 mg rat GHRH(1-29)NH_2 dose per kilogram of body weight per day or lower, in a Sprague Dawley rat submitted to a high-salt diet.

8. The method of claim **1** wherein the oxidative renal damage affects Henle's loop cells.

9. The method of claim **1**, wherein the oxidative damage is due to exaggerated renal medullary osmolality.

10. The method of claim **1** comprising identifying the mammal in need by determining the presence of a marker associated with oxidative renal damage.

11. The method of claim **1** wherein the mammal suffers or is susceptible of suffering from a disease selected from the group consisting of aging- and frailty-related nephropathy and renal failure, diabetes insipidus, diabetes type I, diabetes II, renal disease glomerulonephritis, bacterial or viral glomerulonephritides, IgA nephropathy, Henoch-Schonlein Purpura, membranoproliferative glomerulonephritis, membranous nephropathy, Sjogren's syndrome, nephrotic syndrome minimal change disease, focal glomerulosclerosis and related disorders, acute renal failure, acute tubulointerstitial nephritis, pyelonephritis, genitourinary (GU) tract inflammatory disease, pre-clampsia, renal graft rejection, leprosy, reflux nephropathy, nephrolithiasis, genetic renal disease, medullary cystic, medullar sponge, polycystic kidney disease, autosomal dominant polycystic kidney disease, autosomal recessive polycystic kidney disease, tuberous sclerosis, von Hippel-Lindau disease, familial thin-glomerular basement membrane disease, collagen III glomerulopathy, fibronectin glomerulopathy, Alport's syndrome, Fabry's disease, Nail-Patella Syndrome, congenital urologic anomalies, monoclonal gammopathies, multiple myeloma, amyloidosis and related disorders, febrile illness, familial Mediterranean fever, HIV infection, AIDS, inflammatory disease, systemic vasculitides, polyarteritis nodosa, Wegener's granulomatosis, polyarteritis, necrotizing and crescentic glomerulonephritis, polymyositis-dermatomyositis, pancreatitis, rheumatoid arthritis, systemic lupus erythematosus, gout, blood disorders, sickle cell disease, thrombotic thrombocytopenia purpura, hemolytic-uremic syndrome, acute cortical necrosis, renal thromboembolism, trauma and surgery, extensive injury, burns, abdominal and vascular surgery, induction of anesthesia, side effect of drug abuse or use of including those generating renal oxidative stress and toxicity such as antibiotics and cancer chemotherapeutic agents, malignant disease, adenocarcinoma, melanoma, lymphoreticular, multiple myeloma, circulatory disease, myocardial infarction, cardiac failure, peripheral vascular disease, hypertension, coronary heart disease, non-atherosclerotic cardiovascular disease, atherosclerotic cardiovascular disease, skin disease, psoriasis, systemic sclerosis, respiratory disease, chronic obstructive pulmonary disease, obstructive sleep apnea, hypoxia at high altitude or endocrine disease, acromegaly, diabetes mellitus and conditions related to antibiotic toxicity, infection, inflammation and ischemia.

12. The method of claim **1** wherein the mammal is subjected to chronic hemodialysis.

13. A method of preventing the death of kidney cells due to oxidative stress in a mammal in need thereof, the method comprising administering an effective amount of a ligand to GHRH renal receptor to the mammal.

14. A method of promoting regeneration of kidney cells in a mammal in need thereof, the method comprising administering a ligand to the GHRH renal receptor the mammal.

15. An isolated polypeptide comprising

a. SEQ ID NO.: 13, SEQ ID NO.:16 or SEQ ID NO.:17,

b. a SEQ ID NO.13, SEQ ID NO.:16 or SEQ ID NO.:17 fragment or

c. a SEQ ID NO.:13, SEQ ID NO.:16 or SEQ ID NO.:17 analog;

wherein said polypeptide is free of a N-terminal amino acid sequence of a pituitary GHRH receptor.

16. An antibody capable of specific binding to the polypeptide of claim **15**.

17. An isolated nucleic acid sequence encoding the polypeptide of claim **15**.

18. An isolated nucleic acid selected from the group consisting of

a. a polynucleotide comprising SEQ ID NO.11, SEQ ID NO.12, SEQ ID NO.:18, SEQ ID NO.:19, SEQ ID NO.:20 or SEQ ID NO.:21,

b. a polynucleotide comprising a sequence substantially identical to SEQ ID NO.11, SEQ ID NO.12, SEQ ID NO.:18, SEQ ID NO.:19, SEQ ID NO.:20 or to SEQ ID NO.:21,

c. a polynucleotide comprising a sequence substantially complemetary to a. or b. and;

d. a fragment of any one of a., b. or c.;

wherein the nucleic acid is free of a nucleic acid sequence encoding a N-terminal amino acid sequence of a pituitary GHRH receptor.

19. A vector comprising the nucleic acid sequence of claim **17**.

20. A vector comprising the nucleic acid sequence of claim **18**.

21. An isolated cell expressing the polypeptide of claim **15**.

22. The isolated cell of claim **21** wherein the cell is a renal cell.

23. An assay for identifying a ligand which is capable of specific binding to the polypeptide of claim **15** and not to pituitary GHRH receptor, the assay comprising contacting a test ligand with the polypeptide, measuring binding of the test ligand to the polypeptide and determining the identity of the test ligand.

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专利名称(译)	通过生长激素释放激素和激动剂进行肾脏保护		
公开(公告)号	US20110021429A1	公开(公告)日	2011-01-27
申请号	US12/285282	申请日	2008-10-01
[标]申请(专利权)人(译)	Gaudreau在PIERRETTE THERIAULT KARYNE 贝达德JULIE BOISVERT CHANTALE		
申请(专利权)人(译)	Gaudreau在PIERRETTE THERIAULT KARYNE 贝达德JULIE BOISVERT CHANTALE		
当前申请(专利权)人(译)	Gaudreau在PIERRETTE THERIAULT KARYNE 贝达德JULIE BOISVERT CHANTALE		
[标]发明人	GAUDREAU PIERRETTE THERIAULT KARYNE BEDARD JULIE BOISVERT CHANTALE		
发明人	GAUDREAU, PIERRETTE THERIAULT, KARYNE BEDARD, JULIE BOISVERT, CHANTALE		
IPC分类号	A61K38/25 A61K38/16 A61P13/12 C07K14/00 C07K16/00 C07H21/04 C12N15/63 C12N5/00 G01N33/53		
CPC分类号	A61K38/25 G01N2500/04 C07K14/60 A61P13/12		
优先权	60/960477 2007-10-01 US 61/006057 2007-12-17 US		
外部链接	Espacenet USPTO		

摘要(译)

本发明涉及生长激素释放激素和激动剂的肾脏保护作用。更具体地，本发明涉及用于保护哺乳动物免受氧化性肾损伤，促进有需要的哺乳动物中肾细胞再生和/或防止由于氧化应激导致的肾细胞死亡的方法。本发明还涉及大鼠和人肾GHRH-R序列的鉴定。

Figure 1