(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 September 2006 (14.09.2006)

PCT

(10) International Publication Number
WO 2006/095167 A1

(51) International Patent Classification:
G01N 33/50 (2006.01) *A61K 31/00* (2006.01)
G01N 33/68 (2006.01) *A61K 38/00* (2006.01)
A61K 38/22 (2006.01) *C07K 14/64* (2006.01)

(21) International Application Number:
PCT/GB2006/000823

(22) International Filing Date: 9 March 2006 (09.03.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0504856.6 9 March 2005 (09.03.2005) GB

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

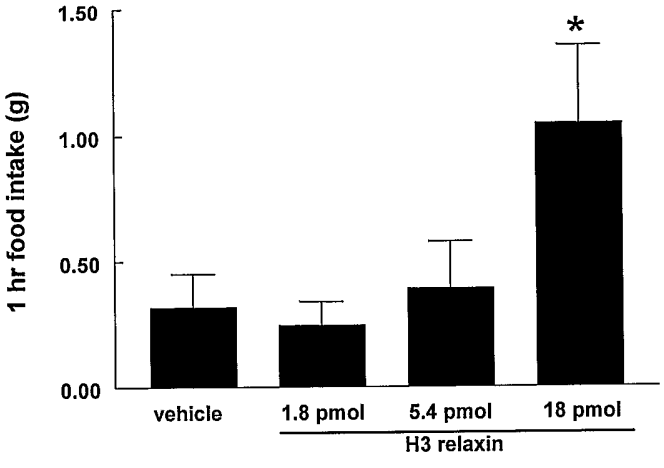
(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:
— *of inventorship (Rule 4.17(iv))*

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) **Title:** APPETITE-INFLUENCING MEDICAMENTS



(57) **Abstract:** The present invention relates to a method for identifying compounds which will be useful as agents for the control of appetite and/or food intake in a mammal, comprising contacting a candidate molecule with GPCR 135 and monitoring the interaction of the candidate molecule and the GPCR135. The invention finds application, amongst others in the field of obesity and cachexia.

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APPETITE-INFLUENCING MEDICAMENTS

Introduction

The present invention relates to agents for use in control of appetite and/or food intake, and to devices and methods for identification of such agents. The invention is applicable in particular, but not solely, in the field of obesity.

Background of the Invention

One of the diseases with the highest incidence but which lacks effective treatment is obesity. It is a debilitating condition which reduces quality of life and substantially increases the risk of other diseases.

In the USA 25% of the adult population is now considered to be clinically obese. It has been estimated that $45 billion of US healthcare costs, or 8% per annum of total healthcare spend, is a direct result of obesity. In Europe the problem is increasing. It was predicted that without new approaches over 20% of the UK population would be clinically obese by 2005. The fact that obesity is a metabolic disease is being increasingly recognised by the medical profession and the health authorities. There is, however, a shortage of effective and safe drugs which can be used in conjunction with diet and exercise for the long-term management of obesity.

Other conditions associated with appetite include those in which the patient is underweight as a result of loss of appetite. The condition anorexia nervosa has become increasingly widespread in the industrial world.

Thus, there is a widespread need for agents both for suppression of and stimulation of appetite.

It is an object of the present invention to provide means to identify and develop further drugs for use in the control of appetite and/or food uptake, and to provide medicaments comprising the drugs.

The relaxin peptides belong to the insulin superfamily, a group of structurally related hormones whose precursors have a domain arrangement similar to that of pro-insulin (Hudson P, Haley J, John M, Cronk M, Crawford R, Haralambidis J, Tregear G, Shine J, Niall H 1983 Structure of a genomic clone encoding biologically active human relaxin. Nature 301:628-631). The insulin superfamily comprises functionally diverse peptides with a common structure: A and B chains with interchain disulphide bridges. Relaxin-1 in mice and rats and the human homologue relaxin-2 were among the first hormones described. Until recently, a single relaxin gene had been described in mice and rats, M1 and R1 respectively, (Soloff MS, Gal S, Hoare S, Peters CA, Hunzicker-Dunn M, Anderson GD, Wood TG 2003 Cloning, characterization, and expression of the rat relaxin gene. Gene 323:149-155; Fowler KJ, Clouston WM, Fournier RE, Evans BA 1991 The relaxin gene is located on chromosome 19 in the mouse. FEBS Lett 292:183-186) and two relaxin genes, H1 and H2, had been described in humans (Hudson P, John M, Crawford R, Haralambidis J, Scanlon D, Gorman J, Tregear G, Shine J, Niall H 1984 Relaxin gene expression in human ovaries and the predicted structure of a human preprorelaxin by analysis of cDNA clones. EMBO J 3:2333-2339). The H2 gene is the homologue of the M1 and R1 genes. The H2 gene product is secreted by the corpus luteum in early pregnancy and primarily associated with female reproductive physiology. It is only recently that an additional relaxin peptide, relaxin-3, and its receptors have been identified. A relaxin gene, relaxin-3, has now been identified in humans (H3) (Bathgate RA, Samuel CS, Burazin TC, Layfield S, Claasz AA, Reytomas IG, Dawson NF, Zhao C, Bond C, Summers RJ, Parry LJ, Wade JD, Tregear GW 2002 Human relaxin gene 3 (H3) and the equivalent mouse relaxin (M3) gene. Novel members of the relaxin peptide family. J Biol Chem 277:1148-1157; hereafter Bathgate et al 2002), mice (M3) and most recently in rats (R3) (Burazin TC, Bathgate RA, Macris M, Layfield S, Gundlach AL, Tregear GW 2002 Restricted, but abundant, expression of the novel rat gene-3 (R3) relaxin in the dorsal tegmental region of brain. J Neurochem 82:1553-1557; hereafter Burazin et al). H3, M3 and R3 retain their insulin-like peptide structure and are highly homologous. Whilst R1 and M1 are expressed in many tissues, R3 and M3 mRNA expression is localised to the nucleus incertus of the brainstem (Burazin et al) which has extensive projections to the hypothalamus (Goto M, Swanson LW, Canteras NS 2001

Connections of the nucleus incertus. J Comp Neurol 438:86-122). Relaxin–like immunoreactivity has been described in the hypothalamic arcuate (ARC) and paraventricular (PVN) nuclei (Bathgate RA, Samuel CS, Burazin TC, Gundlach AL, Tregear GW 2003 Relaxin: new peptides, receptors and novel actions. Trends Endocrinol Metab 14:207-213; hereafter Bathgate et al 2003).

Unlike insulin, relaxin peptides signal via G-protein coupled receptors to modulate intracellular cAMP. R1 and M1 act via two leucine-rich repeat-containing receptors, LGR7 and LGR8 (Hsu SY, Nakabayashi K, Nishi S, Kumagai J, Kudo M, Sherwood OD, Hsueh AJ 2002 Activation of orphan receptors by the hormone relaxin. Science 295:671-674). LGR7, expressed predominantly in reproductive tissues but also in the CNS, binds relaxin-3 with high affinity. However, relaxin-3 is the cognate ligand for two previously orphan G-protein-coupled receptors, GPCR135 and GPCR142 (Liu C, Eriste E, Sutton S, Chen J, Roland B, Kuei C, Farmer N, Jornvall H, Sillard R, Lovenberg TW 2003 Identification of relaxin-3/INSL7 as an endogenous ligand for the orphan G-protein-coupled receptor GPCR135. J Biol Chem 278:50754-50764; Liu C, Chen J, Sutton S, Roland B, Kuei C, Farmer N, Sillard R, Lovenberg TW 2003 Identification of relaxin-3/INSL7 as a ligand for GPCR142. J Biol Chem 278:50765-50770, hereafter Liu et al). Whilst GPCR142 expression is absent in the rat (Chen J, Kuei C, Sutton SW, Bonaventure P, Nepomuceno D, Eriste E, Sillard R, Lovenberg TW, Liu C 2004 Pharmacological Characterization of Relaxin-3/INSL7 Receptors GPCR135 and GPCR142 from Different Mammalian Species. J Pharmacol Exp Ther; hereafter Chen et al), GPCR135 mRNA is highly expressed in the rat brain, particularly the PVN (Liu et al; Chen et al).

Summary of the Invention

The invention is based on the unexpected observation that relaxin-3 can significantly increase food intake in satiated animals. It has been further unexpectedly observed that relaxin-3 has no significant effect on energy expenditure.

The invention provides a method for identifying compounds which will be useful as agents for the control of appetite and/or food intake in a mammal, comprising contacting

a candidate molecule with GPCR135 and monitoring the interaction of the candidate molecule and the GPCR135.

The term "GPCR135" as used herein includes human GPCR135 and peptides having a substantial degree of homology, for example at least 75%, advantageously at least 90%, especially at least 95%, more especially at least 97%, preferably at least 98% and most preferably at least 99% homology, with human GPCR135, and in particular may include GPCR135 of other mammals. Other mammalian GPCR135 having high levels of homology with human GPCR135 includes rat GPCR135 and mouse GPCR135. The term "GPCR135" is further to be understood as including peptides which have a relatively low homology with human GPCR135, for example at least 25% homology, whilst nonetheless substantially retaining the functionality of human GPCR135 in mediating the effects of relaxin-3 on food intake. Furthermore it is to be understood that the term GPCR135 as used herein includes fragments, derivatives and modifications of GPCR135, provided that functionality is retained. The term GPCR135 as used herein is not limited to naturally occurring products but includes synthetically produced GPCR135 (including functionally equivalent fragments, derivatives and modifications) and other synthetically generated peptides that are functionally equivalent to naturally occurring human GPCR135. It will be appreciated that in the foregoing definition "functionally equivalent" refers to substantial equivalence in the function of mediating the effects of relaxin-3 on food intake.

The invention also provides a method for identifying compounds which will be useful in the treatment of obesity, comprising contacting a candidate molecule with GPCR135 and monitoring the interaction of the candidate molecule and the receptor.

Moreover, the invention provides a method for identifying compounds which will be useful as agents for the control of appetite and/or food intake, comprising ascertaining the binding affinity of a candidate molecule to GPCR135 receptor.

The invention further provides the use of a compound which is an antagonist of GPCR135, or a physiologically acceptable salt, solvate or derivative thereof, in the manufacture of a medicament for suppression of appetite and/or food uptake.

The invention also provides the use of a compound which is an agonist of GPCR135, or a physiologically acceptable salt, solvate or derivative thereof, in the manufacture of a medicament for enhancement of appetite and/or food uptake.

Additionally, the invention provides a method for the treatment of an eating disorder in a mammal, including a human, comprising administration of a compound which agonises GPCR135.

Moreover, the invention provides a method for the treatment of obesity in a mammal, including a human, comprising administration of a compound which antagonises GPCR135.

The present invention provides a method for cosmetic weight loss in a mammal, the method comprising administering a composition comprising a GPCR135 antagonist to a mammal. In this circumstance, the weight loss is purely for the purposes of cosmetic appearance.

Suitable tests for determining binding affinity to GPCR135, and for determining agonist and antagonist behaviour are described further below.

Brief Description of the Drawings

Fig. 1A is a graphical illustration of the effect of ICV administration of relaxin-3 (18-180 pmol) in satiated male Wistar rats on 1 h food intake * = $p < 0.05$ vs vehicle;

Fig. 1B is a graphical illustration of the effect of relaxin-3 (18-180 pmol) in satiated male Wistar rats on cumulative food intake over 4 h. & = $p < 0.05$ at 18 pmol vs vehicle, * = $p < 0.05$ at 54 pmol vs vehicle, # = $p < 0.05$ at 180 pmol vs vehicle;

Fig. 2A is a graphical illustration of the effect of iPVN administration of relaxin-3 (1.8-18 pmol) in male Wistar rats on 1 h food intake in early light phase;

Fig. 2B is a graphical illustration of the effect of relaxin-3 (18 pmol) in male Wistar rats on 1 h food intake in early dark phase; * = $p < 0.05$ vs vehicle;

Fig. 3 is a graphical illustration of the effect of IP administration of relaxin-3 (0.03–0.3nmol/g) on 1h food intake in satiated C57BL/6 mice;

Fig. 4 is a graphical illustration of the effect of iPVN administration of equimolar doses of relaxin-3 (H3) and relaxin-2 (H2) on 1 h food intake in satiated male Wistar rats. * = $p < 0.05$ vs vehicle.

Fig. 5 is a graphical illustration of the effect of acute iPVN administration of H3 (180-1620 pmol) on 1 hour food intake in satiated male Wistar rats in the early light phase. * = $p < 0.05$ vs vehicle

Fig. 6 is a graphical illustration of the effect of acute iPVN administration of H3 (540 pmol) on plasma TSH at 15 and 30 minutes post-injection. * = $p < 0.05$ vs vehicle

Fig. 7A is a graphical illustration of the effect of repeated iPVN administration of vehicle or H3 (180 pmol/injection) in *ad libitum* fed rats on 24 hour food intake on day 1 and day 7, * = $p < 0.05$ vs vehicle

Fig. 7B is a graphical illustration of the effect of repeated iPVN administration of vehicle or H3 (180 pmol/injection) in *ad libitum* fed rats on 1 hour food intake in the early light phase on day 1 and day 7, * $p < 0.05$ vs vehicle

Fig. 8 is a graphical illustration of the effect of cumulative food intake after repeated iPVN administration of vehicle, H3 in *ad libitum* fed rats (180 pmol/injection) or H3 in pair-fed (PF) rats for 7 days. White circles = vehicle; plus sign = H3 ad libitum fed group; black triangle = H3 pair fed group, * = $p < 0.05$ vs vehicle

Fig. 9 is a graphical illustration of the effect of repeated iPVN administration of vehicle, H3 in *ad libitum* fed rats (180 pmol/injection) or H3 in pair-fed (PF) for 7 days on plasma TSH, *$p < 0.05$ vs vehicle

Fig. 10 is a graphical illustration of the protein sequences of the human, mouse and rat GPCR135 receptors.

Detailed Description of the Invention

Sequences of GPCR135 are known and documented in the art. The GPCR135 human sequence SEQ ID NO: 1 is as follows:

MQMADAATIATMNKAAGGDKLAELFSLVPDLLEAANTSGNASLQLPDLWWELGLELPDGA
PPGHPPGSGGAESADTEARVRILISVVYWVVCALGLAGNLLVLYLMKSMQGWRKSSINLF
VTNLALTDFQFVLTLPFWAVENALDFKWPFGKAMCKIVSMVTSMNMYASVFFLTAMSVTR
YHSVASALKSHRTRGHGRGDCCGRSLGDSCCFSAKALCVWIWALAALASLPSAIFSTTVK
VMGEELCLVRFPDKLLGRDRQFWLGLYHSQKVLLGFVLPLGIIILCYLLLVRFIADRRAA
GTKGGAAVAGGRPTGASARRLSKVTKSVTIVVLSFFLCWLPNQALTTWSILIKFNAVPFS
QEYFLCQVYAFPVSVCLAHSNSCLNPVLYCLVRREFRKALKSLLWRIASPSITSMRPFTA
TTKPEHEDQGLQAPAPPHAAAEPDLLYYPPGVVVYSGGRYDLLPSSSAY

The GPCR135 rat sequence SEQ ID NO: 2 is as follows:

MPKAHLSMQVASATTAAPMSKAAAGDELSGFFGLIPDLLEVANRSSNASLQLQDLWWELG
LELPDGAAPGHPPGSGGAESADTEARVRILISAVYWVVCALGLAGNLLVLYLMKSKQGWR
KSSINLFVTNLALTDFQFVLTLPFWAVENALDFKWPFGKAMCKIVSMVTSMNMYASVFFL
TAMSVARYHSVASALKSHRTRGHGRGDCCGQSLGESCCFSAKVLCGLIWASAAIASLPNV
IFSTTINVLGEELCLMHFPDKLLGWDRQFWLGLYHLQKVLLGFLLPLSIISLCYLLLVRF
ISDRRVVGTTDGATAPGGSLSTAGARRRSKVTKSVTIVVLSFFLCWLPNQALTTWSILIK
FNVVPFSQEYFQCQVYAFPVSVCLAHSNSCLNPILYCLVRREFRKALKNLLWRIASPSLT
SMRPFTATTKPEPEDHGLQALAPLNATAEPDLIYYPPGVVVYSGGRYDLLPSSSAY

The GPCR135 mouse sequence SEQ ID NO: 3 is as follows:

MQVASATPAATVRKAAAGDELSEFFALTPDLLEVANASGNASLQLQDLWWELGLELPDGA
APGHPPGGGGAESTDTEARVRILISAVYWVVCALGLAGNLLVLYLMKSKQGWRKSSINLF
VTNLALTDFQFVLTLPFWAVENALDFKWPFGKAMCKIVSMVTSMNMYASVFFLTAMSVAR
YHSVASALKSHRTRGRGRGDCCGQSLRESCCFSAKVLCGLIWASAALASLPNAIFSTTIR
VLGEELCLMHFPDKLLGWDRQFWLGLYHLQKVLLGFLLPLSIISLCYLLLVRFISDRRVV
GTTDAVGAAAAPGGGLSTASARRRSKVTKSVTIVVLSFFLCWLPNQALTTWSILIKFNAV

PFSQEYFQCQVYAFPVSVCLAHSNSCLNPILYCLVRREFRKALKNLLWRIASPSLTNMRP
FTATTKPEPEDHGLQALAPLNAAAEPDLIYYPPGVVVYSGGRYDLLPSSSAY

The inventors have shown for the first time that ICV relaxin-3 significantly increases food intake in satiated animals (see Example 1 below). Similarly, relaxin-3 injection into the PVN, an area with a high level of expression of GPCR135, also stimulated food intake and was able to potentiate nocturnal feeding (see Examples 2 and 3 below). Behavioral studies show a significant increase in feeding behavior and no change in other behaviors following iPVN relaxin-3 administration. These studies were performed using human relaxin-3. There is, however, a high level of homology among the relaxin-3 peptides of different species: the mouse and rat peptides are identical and share 92% sequence identity to human relaxin-3 (Chen et al). At the present time, only human relaxin-3 is commercially available but this binds with high affinity to rat GPCR135 (Chen et al).

The inventors have also demonstrated that acute administration of iPVN relaxin-3 has no significant effect on metabolic parameters (see Example 10) and that 7 day repeated iPVN administration of relaxin-3 has no significant effect on the body weight of vehicle-treated animals compared to H3-treated animals pair-fed to the food intake of the vehicle-treated animals (see Example 11). The inventors have therefore shown for the first time that relaxin-3 can significantly increase food intake in animals while having no significant effect on energy expenditure.

In view of their showing of the association between relaxin-3 and feeding behaviour, the inventors propose that the GPCR135 receptor will provide a suitable basis for identifying compounds which have appetite suppressant or appetite stimulating activity. In a preferred embodiment of the invention, candidate molecules for use as agents for control of appetite and/or food intake in a mammal comprises contacting a candidate molecule with GPCR135 and monitoring the effect of the candidate molecule on the activity of GPCR135.

In accordance with certain embodiments of the invention, a method for identifying compounds which will be useful as agents for the control of appetite and/or food intake comprises ascertaining the binding affinity of a candidate molecule to GPCR135, and/or determining whether a candidate molecule is an agonist or an antagonist for GPCR135. Suitable assays for determining the binding affinity, and for determining agonist or antagonist behaviour, in relation to GPCR135 are as follows:

Binding affinity to GPCR135 may be determined by any suitable method, one suitable method being an ELISA assay. An example of an ELISA assay is as described in the following steps:

1. Coat microwells of immuno-detection plate with purified GPCR135 receptor.
2. Incubate at 4°C overnight.
3. Wash unbound receptor off the plate using an appropriate wash solution.
4. Block non-specific binding by adding an appropriate volume of 1% BSA/PBS to each microwell.
5. Incubate at RT for 30-60 minutes.
6. Repeat washing step.
7. Prepare appropriate dilution series of relaxin-3 conjugated to an enzyme such as alkaline phosphatase or horseradish peroxidase.
8. Add appropriate volume of conjugated relaxin-3 to each microwell and incubate for 1 hour.
9. Repeat washing step.
10. Prepare appropriate substrate solution for conjugated enzyme.
11. Add appropriate volume of substrate to each microwell.
12. Incubate at RT for 1 hour.
13. Add stopping solution if appropriate.
14. Read plates on an ELISA plate reader.

Another suitable method for determining binding affinity to GPCR135 is a radioligand saturation binding assay. An example of such an assay is described in the following steps:

1. Transiently transfect appropriate cells (e.g. 293T cells) with a plasmid expressing GPCR135.
2. Plate the transiently transfected cells in a 24-well plate for whole cell binding assay.
3. Remove media and wash with PBS.
4. Pre-incubate cells in binding buffer (e.g. 300µl of 20 mM HEPES, 50 mM NaCl, 1.5 mM $CaCl_2$, 1% BSA, 0.1 mg/ml lysine, 0.01% NaN_4, pH 7.5).
5. Prepare a dilution series of radiolabelled ligand.
6. Add an appropriate amount (e.g. 100 µl) of each dilution of ligand to separate wells of the 24-well plate.
7. Add an excess of radiolabelled ligand to a further well to determine non-specific binding.
8. After incubation, wash cells with PBS, recover cells from the plates using an appropriate amount (e.g. 500 µl) of an appropriate concentration (e.g 1 M) of NaOH and transfer to scintillation vials.
9. Add liquid scintillation mixture to the vials (e.g. Ultima Gold, Packard).
10. Count vials in a liquid scintillation analyser (e.g. Packard 1900 TR).

A GPCR135 agonist is a peptide, small molecule, or chemical compound that preferentially binds to the GPCR135 receptor and stimulates the same biological activity as does relaxin-3. In one embodiment, an agonist for the human GPCR135 receptor binds to the receptor with an equal or greater affinity than human relaxin-3. GPCR135 agonists include relaxin-3 related peptides and peptides that result from natural or synthetic enzymatic or chemical processing of relaxin-3 peptide or a related peptide.

Agonist activity in respect of GPCR135 may be determined by any suitable method, one suitable method being a cell-based assay. For example, such an assay may be a GTPγS-binding assay as described in the following steps:

1. Transfect suitable cells (e.g. CHO-K1) with GPCR135 expression vector.
2. Harvest cells after 2 days and prepare cell membranes by homogenisation in a suitable buffer (e.g. 50 nM Tris-HCl, 5 mM EDTA),
3. Pellet the cell membranes via centrifugation and remove supernatant.
4. Add a suitable GTPγS binding buffer (e.g. 50 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, 10 μM GDP, 1 mM EDTA, pH 8.0 and 100 mM NaCl) and rehomogenise the pellet in the buffer.
5. Add a suitable protease inhibitor to the sample.
6. Prepare a dilution series of the potential agonist and an appropriate negative control.
7. Add each dilution of the potential agonist and negative control to a separate sample of cell membrane expressing GPCR135 in a single well on a 96-well plate.
8. Incubate at RT for 20 min.
9. Add the appropriate amount of ^{35}S-labelled GTPγS to each well to reach a concentration of 200 pM.
10. Allow reaction to proceed for 1 hour at RT.
11. Filter the plate through a 96-well filter plate.
12. Wash with a suitable cold washing buffer (e.g. 50 nM Tris-HCl, pH 7.4, 10 mM $MgCl_2$).
13. Add an appropriate amount of liquid scintillation cocktail (e.g. Microscint-40 from Perkin-Elmer) to each well.
14. Count the plate on an appropriate apparatus.

A GPCR135 antagonist is a peptide, small molecule, or chemical compound that binds to the GPCR135 receptor and inhibits the mediation thereby of the effects of relaxin-3 on food intake. In one embodiment, an antagonist for the human GPCR135 receptor binds to the receptor with an equal or greater affinity than human relaxin-3.

Antagonist activity in respect of GPCR135 may be determined by any suitable method, one suitable method being a cell-based assay. For example, such an assay may be a cAMP-stimulation assay as described in the following steps:

1. Transfect suitable cells (e.g. CHO-K1) with GPCR135 expression vector.
2. Culture cells using an appropriate selection.
3. Seed the receptor expressing cells into 96 well plates at an appropriate density (e.g. 30,000 cells/well).
4. After 24 h, replace the cell culture medium with an appropriate test medium (e.g. Dulbecco's modified Eagle's medium/F-12 (Invitrogen) plus 2 mM isobutylmethylxanthine (Sigma).
5. Add an appropriate amount of test compound to each well.
6. Incubate at RT for 25 min.
7. Add an appropriate amount of 0.5 N HCl to halt the reaction and extract accumulated cAMP.
8. Assay the cAMP concentrations in the extracted media using an appropriate method (e.g. cAMP Flash Plates (Perkin Elmer Life Sciences)).

An alternative example is to assay antagonist activity by performing a competition assay using a modified version of the GTPγS-binding assay described above. In the modified version, both relaxin-3 and a potential antagonist molecule are both added to the wells in step 7. The antagonist activity of the candidate molecule is assessed by measuring its ability to compete with relaxin-3 and thus reduce the GTP-binding activity that is promoted by relaxin-3.

Agonist or antagonist behaviour can be demonstrated in vivo by administering an intracerebroventricular injection of candidate compound in a suitable vehicle, for example 10% acrylonitrile in 0.9% saline, to satiated rats and comparing subsequent food intake at 2 hours and 4 hours following administration with a control group of rats injected with vehicle alone.

The doses of relaxin-3 required to elicit a significant feeding response are in the picomolar range and similar to effective doses of other orexigenic peptides such as ghrelin. For example, a significant orexigenic response with iPVN ghrelin has been seen at 30 pmoles (Wren AM, Small CJ, Abbott CR, Dhillo WS, Seal LJ, Cohen MA, Batterham RL, Taheri S, Stanley SA, Ghatei MA, Bloom SR 2001 Ghrelin causes hyperphagia and obesity in rats. Diabetes 50:2540-2547; hereafter Wren et al) compared to 18 pmoles of H3 relaxin. Similarly, the lowest dose of the potent orexigenic peptide NPY to significantly stimulate feeding in the PVN is 24 pmol (Stanley BG, Kyrkouli SE, Lampert S, Leibowitz SF 1986 Neuropeptide Y chronically injected into the hypothalamus: a powerful neurochemical inducer of hyperphagia and obesity. Peptides 7:1189-1192). As with NPY and ghrelin, the effect of relaxin-3 occurs in the first hour following administration but cumulative food intake remains elevated for several hours.

The present inventors also investigated the maximum orexigenic response elicited by H3. Acute iPVN administration of high dose H3 significantly increased food intake in the first and second hour in satiated rats (180-1620 pmol, see Figure 5), and cumulatively up to 8 hours following injection. The maximum orexigenic response was achieved by a dose of 540 pmol H3 with no further increase at the higher dose of 1620 pmol H3. Although the increase in food intake in the first hour following injection of 540 pmol H3 was almost seven-fold that following vehicle administration, it was only half the orexigenic response seen following administration of an equimolar dose of NPY. However, high dose H3 continued to stimulate food intake for longer than NPY since by the end of the second hour the orexigenic response to H3 almost matched that of NPY.

It is known that several peptides, such as NPY and AgRP, both stimulate food intake and also alter energy expenditure (C.J.Small, Y.L.Liu, S.A.Stanley, I.P.Connoley, A.Kennedy, M.J.Stock, S.R.Bloom, Chronic CNS administration of Agouti-related protein (Agrp) reduces energy expenditure Int.J.Obes.Relat Metab Disord. 2003;27:530-533; C.J.Billington, J.E.Briggs, M.Grace, A.S.Levine, Effects of intracerebroventricular injection of neuropeptide Y on energy metabolism Am.J.Physiol 1991;260:R321-R327; M.Egawa, H.Yoshimatsu, G.A.Bray, Neuropeptide Y suppresses sympathetic activity to

interscapular brown adipose tissue in rats Am.J.Physiol 1991;260:R328-R334.). The inventors also examined the acute effects of H3 on both plasma TSH and other parameters of energy expenditure (VO2, VCO2, RER and ambulatory activity, see Examples 9 & 10). Acute iPVN administration of H3 significantly suppressed plasma TSH at 15 and 30 minutes post injection. However, there were no significant changes in VO2, VCO2, RER or activity when the effects of acute iPVN administration of H3 were examined in the CLAMS system. Energy expenditure is comprised of several components: thyroid hormone activity, BAT activity, activation of the sympathetic nervous system and both locomotor and non-locomotor muscle activity. Thus, although TSH was suppressed, there may be compensation by other components with the net result being no overall change in energy expenditure in response to H3. The inventors have therefore demonstrated that, surprisingly, the effects of acute administration of H3 differ from those of NPY and AgRP in that H3 stimulates food intake without altering energy expenditure.

The inventors also investigated the effects of repeated iPVN administration of H3 on food intake and body weight over a 7 day period (see Example 11). Three groups were studied: 1) vehicle-treated, *ad libitum* fed control animals, 2) H3-treated *ad libitum* fed animals and 3) H3-treated animals pair-fed to the median food intake of the vehicle-treated animals.

Repeated 7-day iPVN administration of H3 significantly increased cumulative food intake in the *ad libitum* fed, H3-treated animals compared with the vehicle-treated *ad libitum* fed controls and cumulative body weight change was increased in the H3-treated group. Further, one hour food intake following injection was significantly increased in the first hour following administration in the early light phase on both day 1 and day 7 with no evidence of tachyphylaxis. These results thus demonstrate that H3 is effective at increasing food intake and increasing body weight over a 7 day period.

Plasma leptin was significantly elevated in *ad libitum* fed H3 treated animals and though epididymal fat pad weight was higher in the *ad libitum* fed H3 treated animals this did not achieve statistical significance.

A comparison of the results from the vehicle-treated control animals and the pair-fed H3-treated animals showed that although there was an increase in cumulative body weight change in the vehicle-treated animals compared to the pair-fed H3-treated animals, this was not significant at the end of the study. This would suggest that there was no significant effect of H3 on energy expenditure and is in keeping with the results of acute H3 administration on metabolic parameters. In keeping with this, there was no difference in BAT UCP-1 mRNA expression between the treatment groups.

There was, however, a significant difference in cumulative body weight change between *ad libitum* fed H3 treated and H3 pair-fed animals by the end of day 7. Again, plasma TSH was significantly suppressed by repeated iPVN injection of H3. In keeping with this, a suppression of TRH release and an increase in SRIF release was seen from hypothalamic slices following administration with 10 nM H3.

The inventors have thus demonstrated that the effects of repeated H3 administration differ from those of other orexigenic peptides such as NPY, AgRP and ghrelin. Repeated administration of NPY, AgRP and ghrelin significantly increases food intake and decreases energy expenditure, with the combined effect resulting in body weight gain. In contrast, while administration of H3 significantly increases food intake, it does not significantly decrease energy expenditure

After repeated iPVN H3 administration, no significant change was observed in testicular or adrenal weight, plasma LH, prolactin or corticosterone. This is in contrast to the effects of chronic NPY administration (A.Sainsbury, I.Cusin, F.Rohner-Jeanrenaud, B.Jeanrenaud, Adrenalectomy prevents the obesity syndrome produced by chronic central neuropeptide Y infusion in normal rats Diabetes 1997;46:209-214; J.Wang,

A.Akabayashi, J.Dourmashkin, H.J.Yu, J.T.Alexander, H.J.Chae, S.F.Leibowitz, Neuropeptide Y in relation to carbohydrate intake, corticosterone and dietary obesity Brain Res. 1998;802:75-88) and leptin deficiency (ob/ob and db/db mice) (J.A.Edwardson, C.A.Hough, The pituitary-adrenal system of the genetically obese (ob/ob) mouse J.Endocrinol. 1975;65:99-107; D.L.Coleman, D.L.Burkart, Plasma corticosterone concentrations in diabetic (db) mice Diabetologia 1977;13:25-26) which results in profound hypercorticosteremia in rodents and alteration in the gonadal axis.

In conclusion, the inventors have demonstrated that acute iPVN administration of high dose H3 increases food intake with similar efficacy to NPY, suppresses plasma levels of TSH but does not alter energy expenditure. The inventors have also shown for the first time that repeated iPVN administration of H3 is effective for 7 days and increases cumulative food intake, body weight and plasma leptin. Repeated iPVN H3 treatment results in a suppression of plasma TSH without alteration in plasma T3. This effect is independent of food intake, since it also occurs in pair-fed animals.

Active agents of the invention having GPCR135 antagonist activity can be used as a prophylaxis to prevent excess weight gain or can be used as a therapeutic to lose excess weight. The excess weight is typically obesity, although the mammal need not be certified as clinically obese in order to be suffering from excess weight. The agent may be in liquid, solid or semi-solid form.

In today's society, the prevention or treatment of excess weight in a mammal is a real need. Preferably the mammal is a human, although it may also include other mammalian animals, such as horses, canine animals (in particular domestic canine animals), feline animals (in particular domestic feline animals) as well as mammals which are produced for meat, such as porcine, bovine and ovine animals. The present invention can be used to prevent excess weight in such animals in order to maximise lean meat production. Throughout this text, the term "prevention" in relation to excess weight means any effect which mitigates any excess weight, to any extent. Throughout this text, the term

"treatment" in relation to excess weight means amelioration of excess weight, to any extent.

Active agents of the invention having GPCR135 agonist activity, including relaxin-3, can be used as a prophylaxis to prevent weight loss or can be used as a therapeutic in the treatment of appetite disorders to promote weight gain. For example, the agents can be used in the treatment of anorexia nervosa.

In another aspect of the invention, an active agent having GPCR135 agonist activity, including relaxin-3, can be used as a therapeutic in the treatment of weight loss due to underlying disease (cachexia). Weight loss due to underlying disease, often termed "cachexia", occurs in patients with a wide variety of diseases including acquired immune deficiency syndrome (AIDS), liver cirrhosis, chronic obstructive pulmonary disease, chronic renal failure, chronic infections including pneumonia, cancer (cancer cachexia), diabetes and heart disease including hypertension and chronic heart failure (CHF) (cardiac cachexia). Cachexia may also occur idiopathically. In all cases, cachexia may be an indicator of a poor prognosis and its reversal, stopping or at least slowing down, is desirable. Indeed, a strong relationship between weight loss and mortality has been found for many conditions.

This aspect of the invention provides a method of treating weight loss due to underlying disease in a patient the method comprising administering to the patient an effective amount of a GPCR135 agonist, a preferred example of which is relaxin-3, which increases appetite, food intake, weight gain, rate of weight gain, or stabilises weight loss, or rate of weight loss.

In treating weight loss due to underlying disease in a patient it is useful if the weight loss is reversed or stopped or at least slowed down. Agonists of GPCR135, a preferred example of which is relaxin-3, are useful for the treatment or prevention of weight loss due to underlying disease (cachexia). These underlying diseases include, for example, but are not restricted to, AIDS, liver cirrhosis, chronic obstructive pulmonary disease with or

without emphysema, chronic renal failure, chronic infections (like pneumonia), cancer (i.e. cancer cachexia), and heart disease including hypertension and chronic heart failure (i.e. cardiac cachexia), and idiopathic cachexia (i.e. cachexia due to unknown disease).

Therapeutic agents according to the invention, that is in particular relaxin-3 or GPCR135 agonists or antagonists, can be provided in the form of a pharmaceutical composition in combination with a pharmaceutically acceptable carrier or diluent. Suitable carriers and/or diluents are well known in the art and include pharmaceutical grade starch, mannitol, lactose, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, (or other sugar), magnesium carbonate, gelatin, oil, alcohol, detergents, emulsifiers or water (preferably sterile). The composition may be a mixed preparation of a composition or may be a combined preparation for simultaneous, separate or sequential use (including administration). The agent can be provided as a crystalline solid, a powder, an aqueous solution, a suspension or in oil.

The compositions according to the invention for use in the aforementioned indications may be administered by any convenient method, for example by oral, rectal, parenteral eg intravenous, intramuscular, or intraperitoneal, mucosal e.g. buccal, sublingual, nasal, subcutaneous or transdermal administration, including administration by inhalation, and the compositions adapted accordingly.

For oral administration, the compositions can be formulated as liquids or solids, for example solutions, syrups, suspensions or emulsions, tablets, capsules and lozenges.

A liquid formulation will generally consist of a suspension or solution of the compound or physiologically acceptable salt in a suitable aqueous or non-aqueous liquid carrier(s) for example water, ethanol, glycerine, polyethylene glycol or an oil. The formulation may also contain a suspending agent, preservative, flavouring or colouring agent.

A composition in the form of a tablet can be prepared using any suitable pharmaceutical carrier(s) routinely used for preparing solid formulations. Examples of such carriers include magnesium stearate, starch, lactose, sucrose and microcrystalline cellulose.

A composition in the form of a capsule can be prepared using routine encapsulation procedures. For example, powders, granules or pellets containing the active ingredient can be prepared using standard carriers and then filled into a hard gelatin capsule; alternatively, a dispersion or suspension can be prepared using any suitable pharmaceutical carrier(s), for example aqueous gums, celluloses, silicates or oils and the dispersion or suspension then filled into a soft gelatin capsule.

Compositions for oral administration may be designed to protect the active ingredient against degradation as it passes through the alimentary tract, for example by an outer coating of the formulation on a tablet or capsule.

Typical parenteral compositions, including compositions for subcutaneous administration, comprise a solution or suspension of the compound or physiologically acceptable salt in a sterile aqueous or non-aqueous carrier or parenterally acceptable oil, for example polyethylene glycol, polyvinyl pyrrolidone, lecithin, arachis oil or sesame oil. Alternatively, the solution can be lyophilised and then reconstituted with a suitable solvent just prior to administration.

Compositions for nasal or oral administration may conveniently be formulated as aerosols, drops, gels and powders. Aerosol formulations typically comprise a solution or fine suspension of the active substance in a physiologically acceptable aqueous or non-aqueous solvent and are usually presented in single or multidose quantities in sterile form in a sealed container, which can take the form of a cartridge or refill for use with an atomising device. Alternatively the sealed container may be a unitary dispensing device such as a single dose nasal inhaler or an aerosol dispenser fitted with a metering valve which is intended for disposal once the contents of the container have been exhausted. Where the dosage form comprises an aerosol dispenser, it will contain a pharmaceutically

acceptable propellant. The aerosol dosage forms can also take the form of a pump-atomiser.

Compositions suitable for buccal or sublingual administration include tablets, lozenges and pastilles, wherein the active ingredient is formulated with a carrier such as sugar and acacia, tragacanth, or gelatin and glycerin.

Compositions for rectal or vaginal administration are conveniently in the form of suppositories (containing a conventional suppository base such as cocoa butter), pessaries, vaginal tabs, foams or enemas.

Compositions suitable for transdermal administration include ointments, gels, patches and injections including powder injections.

Conveniently the composition is in unit dose form such as a tablet, capsule or ampoule.

Relaxin-3 and other therapeutic agents according to the invention may be administered peripherally at a dose of, for example, 0.01 nmoles or more per kg body weight of the subject, for example, 0.02 nmoles or more, for example, 0.05 nmoles or more, for example, 1 nmole or more, for example, 2 nmoles or more, up to 12 nmoles per kg body weight. The amount used may be up to 5 nmoles, for example, up to 4 nmoles, for example, up to 3 nmoles, for example, up to 2 nmoles, for example, up to 1 nmoles, for example, up to 0.5 nmoles, for example, up to 0.4 nmoles, for example, up to 0.2 nmoles per kg body weight. The dose is generally in the range of from 0.01 to 12 nmoles per kg body weight, for example, within any combination of upper and lower ranges given above. A dose may be calculated on an individual basis or on the basis of a typical subject, often a 70 or 75 kg subject. Dosages may alternatively be calculated on the basis of body surface area. The dose may be administered before each meal.

For subcutaneous administration, a dose of therapeutic agent within the range of from 10 nmol to 500 nmol, which dose is calculated on the basis of a 75 kg subject, may be administered, generally before meals.

A pharmaceutical preparation in unit dosage form for peripheral administration preferably comprises an amount of therapeutic agent calculated on the basis of the per kg doses given above. Typically, the dose may be calculated on the basis of a 70 or 75 kg subject. A composition for subcutaneous administration, for example, may comprise a unit dose of therapeutic agent within the range of from 10 nmol to 500 nmol, calculated on the basis of a 75 kg subject.

It is preferable to administer the agent via a peripheral route of administration, that is to say, via a route other than directly to the brain. Examples of such routes include oral, rectal, parenteral e.g. intravenous, intramuscular, or intraperitoneal, mucosal e.g. buccal, sublingual, nasal, subcutaneous or transdermal administration, including administration by inhalation. Unlike relaxin-1, relaxin-3 is expressed in few peripheral tissues and only at low levels. Whilst peripheral administration of relaxin-1 is able to elicit central effects, such as drinking, initial indications are that peripheral administration of relaxin-3 did not alter food intake (see Example 4 below). In the case of relaxin-3, and of other agents that are peptides, injection into the paraventricular nucleus or intracerebroventricular injection has been shown to be advantageous. Based on that observation, preferred methods of administration will be those which are effective in delivery of the active agent into the brain. In the case of some active agents, in particular those which are small molecules, that will be achievable by means of peripheral administration of the molecule per se or a prodrug. For certain other active agents, for example polypeptides, peripheral administration will be less preferred.

The present invention provides a pharmaceutical composition comprising a therapeutic agent according to the invention and a pharmaceutically suitable carrier, in a form suitable for oral, rectal, parenteral eg intravenous, intramuscular, or intraperitoneal, mucosal e.g. buccal, sublingual, nasal, subcutaneous or transdermal administration,

including administration by inhalation. If in unit dosage form, the dose per unit may be calculated on the basis of the per kg doses given above.

If desired, one or more other agents, such as, but not limited to, an additional appetite suppressant, may also be administered. Specific, non-limiting examples of an additional appetite suppressant include amfepramone (diethylpropion), phentermine, mazindol and phenylpropanolamine, fenfluramine, dexfenfluramine, and fluoxetine.

When used in combination with another agent, active compounds of the invention may be administered simultaneously or substantially simultaneously as the other agent, or sequentially, in either order. Active compounds of this invention and the other agent may be administered in a single pharmaceutical composition or in separate compositions, and they may be administered by the same route or by different routes. It is generally more convenient to administer all the active agents in a single composition. However, in some cases it may be necessary or appropriate to administer the active agents by different routes. For example, peptides are generally not stable on oral administration unless modified or formulated in a special way, so must generally be administered via a non-oral route. Some agonists are chemical compounds that are stable when administered orally. It may be appropriate to administer active agents of the invention non-orally and the other component by a non-oral route or vice versa.

All preferred features given above also apply to the application of the invention in cosmetic weight loss.

In contrast to relaxin-3, equimolar doses of human relaxin-2 did not elicit any increase in feeding (see the Example 5 below). Since both relaxin-2 and relaxin-3 bind LGR7 receptors with high affinity but only relaxin-3 binds GPCR135 with similar affinity, this would suggest the GPCR135 receptor may mediate the effects of relaxin-3 on food intake. In keeping with this, neither relaxin-1 null mice nor LGR7 null mice have any reported abnormality of gross phenotype, such as body weight (Kamat AA, Feng S,

Bogatcheva NV, Truong A, Bishop CE, Agoulnik AI 2004 Genetic targeting of relaxin and insulin-like factor 3 receptors in mice. Endocrinology 145:4712-4720).

The action of some orexigenic peptides, for example ghrelin, is mediated via NPY, AgRP and the melanocortin system (Chen HY, Trumbauer ME, Chen AS, Weingarth DT, Adams JR, Frazier EG, Shen Z, Marsh DJ, Feighner SD, Guan XM, Ye Z, Nargund RP, Smith RG, Van der Ploeg LH, Howard AD, MacNeil DJ, Qian S 2004 Orexigenic action of peripheral ghrelin is mediated by neuropeptide Y and agouti-related protein. Endocrinology 145:2607-2612). Central administration of ghrelin upregulates the expression of NPY and AgRP mRNA in the hypothalamus after 4 hours (Shintani M, Ogawa Y, Ebihara K, Aizawa-Abe M, Miyanaga F, Takaya K, Hayashi T, Inoue G, Hosoda K, Kojima M, Kangawa K, Nakao K 2001 Ghrelin, an endogenous growth hormone secretagogue, is a novel orexigenic peptide that antagonizes leptin action through the activation of hypothalamic neuropeptide Y/Y1 receptor pathway. Diabetes 50:227-232; Kamegai J, Tamura H, Shimizu T, Ishii S, Sugihara H, Wakabayashi I 2000 Central effect of ghrelin, an endogenous growth hormone secretagogue, on hypothalamic peptide gene expression. Endocrinology 141:4797-4800). In contrast, relaxin-3 did not alter hypothalamic NPY, POMC or AgRP mRNA expression. This suggests that the effects of relaxin-3 on food intake may be mediated by an unknown mechanism or act downstream of NPY, AgRP and POMC.

In summary, these results suggest that ICV and PVN administration of relaxin-3 stimulate feeding in male rats and that this effect is mediated via the GPCR135 receptor. The mechanism for the orexigenic action of relaxin-3 remains to be established but does not appear to be via regulation of hypothalamic NPY, POMC or AgRP.

The present invention is now described by way of example only in the following non-limiting Examples.

Materials and Methods

Materials

Human relaxin-3 (H3) used in the Examples was purchased from Phoenix Pharmaceuticals (Belmont, CA) and human relaxin-2 (H2) from Dr A. Parlow, National Hormone and Peptide Program (Torrance, CA). Reagents for Ribonuclease Protection Assay studies were purchased from Ambion (Austin, TX). Reagents for hypothalamic explant studies were supplied by BDH (Poole, Dorset, UK).

Animal studies

Male Wistar rats (250-300 g) were maintained in individual cages for all studies. Intraperitoneal (IP) feeding studies were performed in C57BL/6 mice (25-30 g) maintained in individual cages.

All animals were kept under controlled temperature (21-23°C) and light (12 h light, 12 h dark, lights on at 0700 h) with *ad libitum* access to food (RM1 diet, SDS, UK) and water. All procedures undertaken were approved by the British Home Office Animals Scientific Procedures Act 1986 (project license 70/5516).

Food intake studies

Male Wistar rats underwent third ventricle (ICV) or intra-paraventricular nucleus (iPVN) cannulation 7-10 days before feeding studies and were habituated to regular handling and injection, as previously described (Wren et al). Central injections (5μl (ICV) or 1μl (iPVN)) were administered via stainless steel injectors (27-gauge (ICV) or 31-gauge (iPVN)), placed in and projecting 1 mm below the end of the cannula, over 1 minute. IP injections were given in a volume of 100 μl. All compounds were dissolved in vehicle (10% acetonitrile in 0.9% saline) and studies were performed in satiated rats (n = 10-12) or mice (n = 9-10) in the early light phase (0900 – 1000 h) unless otherwise stated. Following injection, animals were returned to their home cage with pre-weighed chow. Food intake was measured at 1, 2, 4, 8, and 24 hours post injection.

IntraPVN cannula position was verified histologically at the end of the study (Wren et al). Immediately following decapitation, 1 μl Indian ink was injected into the cannula. The brains were removed and fixed in 4% paraformaldehyde, dehydrated in 40% sucrose, frozen and stored at –70°C. Brains were sliced on a cryostat (Bright, Huntingdon, UK)

into 15 μm coronal sections and correct PVN placement determined by microscopy according to the position of the Indian ink. ICV cannula position was verified by a positive dipsogenic response to angiotensin II (150 ng/rat). Only those animals with correct cannula placement were included in the data analysis.

Behavioral response following iPVN administration of relaxin-3

Behavioral responses were monitored following iPVN administration of vehicle, 18 pmol or 180 pmol relaxin-3 (H3). Animals were immediately returned to their home cages and observed for an hour following injection by an investigator blinded to the treatment. Behavior was classified into one of nine categories. Each rat was observed for three 3 sec periods every 6 min and the behavior in each period scored as previously described (Kong WM, Martin NM, Smith KL, Gardiner JV, Connoley IP, Stephens DA, Dhillo WS, Ghatei MA, Small CJ, Bloom SR 2004 Triiodothyronine Stimulates Food Intake via the Hypothalamic Ventromedial Nucleus Independent of Changes in Energy Expenditure. Endocrinology 145:5252-5258; hereafter Kong et al).

Hypothalamic neuropeptide expression following relaxin-3 administration

Hypothalamic neuropeptide mRNA expression was assessed following ICV administration of vehicle or relaxin-3 (H3) (180pmol). Food was removed immediately following injection and at four hours animals were killed, hypothalami dissected and snap frozen. Hypothalamic neuropeptide Y (NPY), agouti related peptide (AgRP) and pro-opiomelanocortin (POMC) mRNA expression were determined by ribonuclease protection assay (RPA). Briefly, RNA was extracted using Tri-Reagent (Helena Biosciences, Sunderland) according to the manufacturer's protocol. Rat β-actin (Ambion Inc.) was used to correct for RNA loading. RNA was hybridized overnight at 42°C with 1.3×10^3 Bq of ^{32}P[CTP] labelled riboprobe. Reaction mixtures were digested with RNase A/T1, the protected fragments precipitated and separated on a 4% polyacrylamide gel. The dried gel was exposed to a phosphorimager screen overnight and bands quantified by image densitometry using ImageQuant software (Molecular Dynamics, Sunnyvale, CA) (Kong et al).

Acute effects of high dose iPVN relaxin-3 administration on food intake

Male Wistar rats (n = 10–12) received an iPVN injection of vehicle or increasing doses of H3 (180 pmol, 540 pmol and 1620 pmol). The lowest dose of H3 used was 10 fold higher than that used in previous iPVN injection studies (B.M.McGowan, S.A.Stanley, K.L.Smith, N.E.White, M.M.Connolly, E.L.Thompson, J.V.Gardiner, K.G.Murphy, M.A.Ghatei, S.R.Bloom, Central relaxin-3 administration causes hyperphagia in male Wistar rats Endocrinology 2005;146:3295-3300). For comparison, one group received the potent orexigen, NPY (500 pmol/animal iPVN). Following injection, animals were returned to their home cage with pre-weighed chow. Food intake was measured at 1, 2, 4, 8, and 24 hours post-injection.

Acute effects of high dose iPVN relaxin-3 administration on plasma TSHs

Male Wistar rats (n = 9-11) received a single injection of vehicle or H3 (540 pmol). Fifteen or 30 minutes following administration, animals were killed by decapitation and plasma was collected into plastic lithium heparin tubes containing 4200 KIU aprotinin (Bayer, Haywards Heath, UK). Plasma was separated by centrifugation, frozen and stored at -70°C until radioimmunoassay (RIA) for thyroid stimulating hormone (TSH).

Acute effects of iPVN relaxin-3 administration on measurements of energy expenditure

Metabolic parameters were measured following a single iPVN injection of vehicle or H3 (180 pmol) (n = 12 per group) in the early light phase (0900 h – 1000 h) by indirect calorimetry using an open-circuit Oxymax system of the Comprehensive Lab Animal Monitoring System (CLAMS; Columbus instruments, Columbus, OH). Animals were individually housed in plexiglass cages through which air was passed at a flow rate of 2.5L/min. All rats were acclimatized to the cages for three days before the injection of vehicle or H3. Body weight was measured during the three day acclimatization period and the morning of the injection. Animals were maintained at 24°C with a 12:12hr light-dark cycle (light period 0700-1900). Food (ground RM1 chow) and water were freely available and food was automatically weighed at 30 minute intervals. Exhaust air from each air tight chamber was sampled for one minute at 30 minute intervals. O_2 consumption and CO_2 production values were normalised with respect to body weight.

The ambulatory activity of each animal was assessed simultaneously using the optical beam technique by an Opto M3 (Columbus Instruments). Consecutive photo-beam breaks were scored as an ambulatory movement. Cumulative activity counts were recorded for each 30 minute interval. Heat production was calculated by deriving a calorific value (CV) based on the observed respiratory exchange ratio (RER). This calorific value was then used with the observed oxygen consumption (VO_2) to calculate heat, expressed per gram lean body weight (cal/h/g BW). The effects of vehicle or H3 on metabolic indices were analyzed by determining the increment from baseline for each parameter.

Effects of 7 day repeated iPVN administration of relaxin-3 on food intake and body weight

Male Wistar rats were randomized to one of three experimental groups (n= 8-11): 1) vehicle – animals received twice daily injections of vehicle (0900 h and 1900 h) for 7 days with *ad libitum* access to food and water, 2) H3 *ad libitum* fed group – rats received twice daily injections of H3 (180 pmol) for 7 days with *ad libitum* access to food and water, 3) H3 pair-fed group – rats received twice daily injections of H3 (180 pmol) for 7 days and were pair-fed to the median food intake of the vehicle group in the equivalent period 24 hours previously.

The dose of 180 pmol H3 was chosen as the lowest dose which gave a highly significant feeding response when administered acutely in this study. The injection interval was chosen based on a significant increase in cumulative food intake up to 8 hours but absent at 24 hours following injection during the early light phase.

Body weight was measured daily at 0900 h. Food was weighed immediately before and 1 hour after each injection to allow calculation of cumulative food intake and food intake in the first hour in response to each injection. Animals that lost more than 10 g in body weight over the course of the study were excluded. A final food and body weight measurement was taken at 0900 h on day 8.

Effects of 7 day repeated iPVN administration of relaxin-3 on fat mass, plasma hormones and UCP-1 expression

Rats from example 9 were killed by decapitation on day 8 at 0900-1000 h, and plasma was collected into plastic lithium heparin tubes containing 4200 KIU aprotinin. Plasma was separated by centrifugation, frozen and stored at -70°C until RIA. Plasma was assayed for pituitary hormones including TSH, prolactin, luteinizing hormone (LH), growth hormone (GH), cortisol and leptin. Weights of epididymal fat pads (white adipose tissue or WAT), interscapular brown adipose tissue (BAT), adrenals and testes were determined.

Expression of UCP-1 mRNA in interscapular brown adipose tissue was assessed by northern blot. RNA was extracted from BAT using Tri-reagent (Helena Biosciences, Sunderland, UK) according to the manufacturer's protocol. Northern Blot analysis was performed as previously described (W.S.Dhillo, C.J.Small, J.V.Gardiner, G.A.Bewick, E.J.Whitworth, P.H.Jethwa, L.J.Seal, M.A.Ghatei, J.P.Hinson, S.R.Bloom, Agouti-related protein has an inhibitory paracrine role in the rat adrenal gland Biochem.Biophys.Res.Commun. 2003;301:102-107). Five micrograms RNA was size-separated on a denaturing MOPS/formaldehyde gel (1% agarose) and transferred to a Hybond-N membrane (GE Healthcare, Slough, UK). The RNA was fixed by baking at 80°C for 2 hours before probing with a riboprobe corresponding to nucleotides 351-658 of the rat UCP-1 cDNA sequence (accession number M11814). The [α-^{32}P] dCTP labelled probe was denatured and then hybridized overnight at 55°C in a mixture of 2.5 mM EDTA pH 8, 0.5% dried milk, 0.25 M sodium phosphate buffer (pH 7.2), 5% SDS, 25 μM aurin tricarboxylic acid. Non-specific hybridization was removed by increasingly stringent washes, the final one being 0.1 x SSC/0.1% (w/v) SDS at 60°C for 30 minutes. The filter was exposed to phosphoscreen overnight prior to quantification of UCP-1 mRNA expression using ImageQuant software (GE Healthcare, Chalfont St Giles, UK). Blots were reprobed with oligo(dT)$_{12-18}$ to enable differences in RNA loading to be corrected.

Effect of relaxin-3 on in vitro release of hypothalamic neuropeptides

The static incubation system was used as previously described (S.A.Stanley, C.J.Small, M.S.Kim, M.M.Heath, L.J.Seal, S.H.Russell, M.A.Ghatei, S.R.Bloom, Agouti related peptide (Agrp) stimulates the hypothalamo pituitary gonadal axis in vivo & in vitro in male rats Endocrinology 1999;140:5459-5462, hereafter Stanley et al). Briefly, male Wistar rats were killed by decapitation and the brain immediately removed. The brain was mounted with ventral surface uppermost and placed in a vibrating microtome (Microfield Scientific Ltd., Dartmouth, UK). A 1.7 mm slice to include the PVN was taken from the basal hypothalamus and incubated in individual chambers containing 1 ml artificial cerebrospinal fluid (aCSF) (20 mM $NaHCO_3$, 126 mM NaCl, 0.09 mM Na_2HPO4, 6 mM KCL, 1.4 mM $CaCl_2$, 0.09 mM $MgSO_4$, 5 mM glucose, 0.18 mg/ml ascorbic acid and 100 µg/ml aprotinin), equilibrated with 95% O_2 and 5% CO_2. The tubes were placed on a shaking platform in a water bath maintained at 37°C. After an initial 2 h equilibration period, the hypothalami were incubated for 45 min in 600 µl aCSF (basal period) before being challenged with H3 (10 nM) for 45 minutes. Finally, the viability of the tissue was verified by a 45 min exposure to 56 mM KCl; isotonicity was maintained by substituting K^+ for Na^+. At the end of each period, the aCSF was removed and frozen at -20°C until measurement of hypothalamic hormones [Thyrotropin releasing hormone (TRH), Somatotropin release inhibitory factor (SRIF)] by RIA.

Radioimmunoassays

Plasma pituitary hormone concentrations were assayed using reagents and methods provided by the National Institute of Diabetes and Digestive Diseases and the National Hormone and Peptide Program (Dr. A. Parlow, Torrance, CA), as previously described (Stanley et al; M.Desai, C.D.Byrne, K.Meeran, N.D.Martenz, S.R.Bloom, C.N.Hales, Regulation of hepatic enzymes and insulin levels in offspring of rat dams fed a reduced-protein diet Am.J.Physiol 1997;273:G899-G904; J.F.Todd, C.J.Small, K.O.Akinsanya, S.A.Stanley, D.M.Smith, S.R.Bloom, Galanin is a paracrine inhibitor of gonadotroph function in the female rat Endocrinology 1998;139:4222-4229; M.S.Kim, C.J.Small, S.A.Stanley, D.G.Morgan, L.J.Seal, W.M.Kong, C.M.Edwards, S.Abusnana, D.Sunter, M.A.Ghatei, S.R.Bloom, The central melanocortin system affects the hypothalamo-

pituitary thyroid axis and may mediate the effect of leptin J.Clin.Invest 2000;105:1005-1011). Hypothalamic hormones (SRIF, TRH) were assayed using in-house radioimmunoassays (RIAs) as previously described (K.O.Akinsanya, M.A.Ghatei, S.R.Bloom, Gonadal steroids regulate rat anterior pituitary levels of TSH-releasing hormone- and pyroglutamyl-glutamyl-proline amide-like immunoreactivity Endocrinology 1995;136:734-740; A.M.Wren, C.J.Small, C.V.Fribbens, N.M.Neary, H.L.Ward, L.J.Seal, M.A.Ghatei, S.R.Bloom, The hypothalamic mechanisms of the hypophysiotropic action of ghrelin Neuroendocrinology 2002;76:316-324). Leptin (Linco Research, Inc, St. Charles, MO), corticosterone (MP Biomedicals, Irvine, CA) and free T3 (EuroDPC Ltd, Gwynedd, UK) were measured using commercially available RIA kits.

Statistical analysis

Results are shown as mean ± S.E.M. Data from acute feeding studies, plasma hormone levels and neuropepride expression were compared by ANOVA with *post-hoc* Dunnett's test or with *post-hoc* LSD test (Systat, Evanston, IL). Neuropeptide expression data were compared by unpaired Student's t-test between control and treated groups. Behavioral data were non-parametric and are expressed as median number of occurrences of behavior (interquartile ranges are expressed in square brackets). Comparison between groups was made by Mann-Whitney U test. Cumulative food intake and body weight data from the repeated injection study was analyzed using marginal models with exchangeable correlation matrix and robust standard errors (Stata 8, Statacorp LP, TX). Energy expenditure parameters were analyzed by unpaired Student *t*-test between control and treated group at each time interval. Hypothalamic explant data was compared by paired Student *t*-test between control and treated groups. In all cases, $p < 0.05$ was considered to be statistically significant.

Examples

Feeding studies

To investigate the hypothesis that relaxin-3 is involved in regulation of appetite, food intake was determined following central or peripheral relaxin-3 administration.

Example 1: Effect of ICV relaxin-3 on food intake in satiated rats

Animals received an ICV injection of vehicle or relaxin-3 (18, 54 or 180 pmol H3). Doses used were based on previously reported effects of porcine relaxin-1 on water intake (Thornton SM, Fitzsimons JT 1995 The effects of centrally administered porcine relaxin on drinking behaviour in male and female rats. J Neuroendocrinol 7:165-169). ICV relaxin-3 significantly increased food intake in the first hour at both 54 pmol and 180 pmol [0.96 ± 0.16 g (vehicle) vs 1.80 ± 0.27 g (54 pmol H3) and 1.81 ± 0.21 g (180 pmol H3), $p < 0.05$] (Fig 1A). There was no significant difference in interval food intake between control and treated groups at later time points. However, cumulative food intake was significantly increased at all doses of relaxin-3 at 2 h and 4 h following ICV administration [1.38 ± 0.29 g (vehicle) vs 2.39 ± 0.29 g (18 pmol H3), 2.37 ± 0.31 g (54 pmol H3) and 2.28 ± 0.25 g (180 pmol H3) at 2 h and 1.76 g ± 0.29 g (vehicle) vs 2.73 ± 0.35 g (18 pmol H3), 2.95 ± 0.30 g (54 pmol H3) and 2.67 ± 0.29 g (180pmol H3) at 4 h, $p < 0.05$] (Fig 1B). There was no effect on water intake following ICV H3 relaxin administration (data not shown).

Example 2: Effect of iPVN relaxin-3 on food intake in satiated rats

Animals received an iPVN injection of either vehicle or relaxin-3 (1.8, 5.4 or 18 pmol H3). Doses used were ten-fold less than those eliciting a feeding response following ICV administration (Wren et al). Intra-PVN relaxin-3 administration significantly increased food intake in the first hour at 18 pmol [0.34 ± 0.16 g (vehicle) vs 1.23 ± 0.30 g, $p < 0.05$] (Fig 2A). There was no significant difference in interval food intake but cumulative food intake was significantly increased at 2, 4, 8 and 24 hours following iPVN administration of 18 pmol relaxin-3 [0.38 ± 0.18 g (vehicle) vs 1.49 ± 0.31 g at 2 h, 0.63 ± 0.27 g (vehicle) vs 1.61 ± 0.35 g at 4h, 1.24 ± 0.35 g (vehicle) vs 2.84 ± 0.61 g at 8 h and 25.9 ± 1.18 g (vehicle) vs 30.0 ± 1.1 g at 24 h, $p < 0.05$].

Example 3: Effect of iPVN relaxin-3 on dark phase food intake

Rats received an iPVN injection of either vehicle or relaxin-3 (18 pmol H3) at the beginning of the dark phase. Nocturnal food intake was significantly increased in the

first hour following relaxin-3 administration [4.43 ± 0.32 g (vehicle) vs 6.57 ± 0.42 g, $p < 0.05$] (Fig 2B). There was no significant effect on interval food intake at later time points but cumulative food intake was significantly increased in relaxin-3-treated animals for 4 hours following administration in the early dark phase [9.68 ± 0.60 g (vehicle) vs 12.28 ± 0.76 g, $p < 0.05$].

Example 4: Effect of IP relaxin-3 on food intake

Satiated mice received an IP injection of either vehicle or increasing doses of relaxin-3 (0.03, 0.01 or 0.3 nmol/g H3). Doses of relaxin-3 used were equivalent to effective doses of the orexigenic peptide ghrelin following peripheral administration. Peripheral administration of relaxin-3 did not alter food intake in satiated mice at any time point following injection [0.06 ± 0.01 g (vehicle) vs 0.034 ± 0.02 g (0.3nmol/g H3)] (Fig 3).

Example 5: Effect of iPVN administration of relaxin-2 on food intake in satiated rats

To differentiate the receptor mediating the effects of relaxin-3 on food intake, the feeding response to relaxin-3, which binds both LGR7 and GPCR135 receptors, was compared to that following administration of relaxin-2 (H2), which binds LGR7 but not GPCR135. Satiated rats received an iPVN injection of either 1.8-18 pmol H3 or 1.8-18 pmol H2. Following iPVN administration of equimolar doses, relaxin-3 stimulated one-hour food intake as previously shown in Example 2 [0.27 ± 0.11 g (vehicle) vs 1.52 ± 0.51 g (18 pmol H3), $p < 0.05$]. In contrast, relaxin-2 had no significant effect on food intake at any time point following administration [0.27 ± 0.11 g (vehicle) vs 0.14 ± 0.04 g (18 pmol H2), $p < 0.05$] (Fig 4).

Example 6: Behavioral response following acute iPVN administration of relaxin-3

Feeding behavior was significantly increased following iPVN administration of relaxin-3 (180 pmol H3). There were no significant differences in other behaviors and there were no abnormal behaviors following an injection of relaxin-3 (Table 1).

Table 1: Effect of iPVN administration of relaxin-3 (18 pmol or 180 pmol) on behavior in the first hour following injection. * = $p < 0.05$ vs vehicle

Behavior	Vehicle	Relaxin-3 (18pmol H3)	Relaxin-3 (180pmol H3)
Feeding	2 [0-4]	2 [2-5]	6 [4-7] *
Drinking	0 [0]	0 [0]	0 [0]
Grooming	7 [1-8]	6.5 [4.25 -8]	6 [5-6]
Burrowing	0 [0]	0 [0]	0 [0]
Rearing	8 [3-10]	5 [4-9.75]	6 [4-8]
Locomotion	4 [2-4]	3 [3-4]	3 [1-5]
Sleep	0 [0-3]	0 [0-2.25]	0 [0-8]
Head down	10 [5-19]	14.5 [6.5-16]	9 [6-9]
Tremor	0 [0]	0 [0-1]	0 [0]

Example 7: Hypothalamic neuropeptide mRNA expression

Following an ICV injection of 180 pmol H3, there was no difference in hypothalamic NPY, AgRP or POMC mRNA expression 4 hours post injection compared to vehicle treated animals [NPY: 26.8 ± 1.26 (vehicle) vs 27.8 ± 2.90 (H3). AgRP: 13.1 ± 1.35 (vehicle) vs 13.0 ± 0.78 (H3). POMC: 1.90 ± 0.17 (vehicle) vs 1.85 ± 0.24 (H3), units are arbitrary, $p< 0.05$].

Example 8: Acute effects of high dose iPVN relaxin-3 administration on food intake

A single iPVN injection of high dose human relaxin-3 (H3) to satiated male Wistar rats significantly increased food intake in the first hour post-administration at all doses [0-1 hour food intake: 0.4 ± 0.1 g (vehicle) vs 1.6 ± 0.5 g (180 pmol H3), 2.4 ± 0.5 g (540 pmol H3), and 2.2 ± 0.5 g (1620 pmol H3), $p< 0.05$ for all doses vs vehicle] (Figure 5). The effect was also significant at these doses in the second hour post-injection [1-2 hour food intake: 0.2 ± 0.1 g (vehicle) vs 2.2 ± 0.7 g (180 pmol H3), 2.6 ± 0.4 g (540 pmol H3), and 2.1 ± 0.5 g (1620 pmol H3), $p< 0.05$ for all doses vs vehicle]. There was no significant difference in interval food intake between control and treated groups at later time points. Cumulative food intake was significantly increased 2, 4 and 8 hours post

iPVN administration of 180, 540 and 1620 pmol H3 [0-8 hour food intake: 2.7 ± 0.7 g (vehicle) vs 5.6 ± 1.0 g (180 pmol H3), 6.3 ± 0.6 g (540 pmol H3), and 6.9 ± 0.6 g (1620 pmol H3), $p < 0.05$ for all doses vs vehicle].

Although the maximum orexigenic effect of H3 (540 pmol) increased food intake almost seven-fold in the first hour, this response was equivalent to approximately 50% of the increase in food intake achieved by iPVN injection of NPY (500 pmol). However, by the end of the second hour, the increase in food intake with H3 was approximately 90% of that induced by NPY [0-1 hour food intake: 2.4 ± 0.g (540 pmol H3) vs 5.2 ± 1.2 g (500 pmol NPY) and 0-2 hour food intake: 5.0 ± 0.7 g (540 pmol H3) vs 5.8 ± 1.3 g (500 pmol NPY) 2 hours post-injection].

Example 9: Acute effects of high dose iPVN relaxin-3 administration on thyroid stimulating hormone

IntraPVN administration of H3 (540 pmol) significantly reduced plasma thyroid stimulating hormone (TSH) at both 15 and 30 minutes following injection compared to vehicle [2.46 ± 0.34 ng/ml (vehicle) vs 1.58 ± 0.18 ng/ml (H3) at 15 min, $p < 0.05$ vs vehicle and 3.88 ± 0.44 ng/ml (vehicle) vs 2.48 ± 0.26 ng/ml (H3) at 30 min, $p < 0.05$ vs vehicle] (Figure 6).

Example 10: Acute effects of iPVN relaxin-3 administration on measurements of energy expenditure

A single iPVN injection of H3 (180 pmol) did not have a significant effect on the increment in VO2 although the trend was slightly higher for H3 treated animals [mean VO2 consumption for the first 8 hours post-injection: 1296.6 ± 66.4 ml/kg/min (vehicle) vs 1348.2 ± 59.7 ml/kg/min (H3)]. VCO2, RER and physical activity did not differ when compared to vehicle. As previously observed, a significant increase in food intake was seen in the first hour after H3 administration (data not shown).

Example 11: Effects of 7 day repeated iPVN administration of relaxin-3 on food intake and body weight

Three groups were studied: 1) vehicle treated, *ad libitum* fed, 2) H3 treated, *ad libitum* fed and 3) H3 treated pair-fed to the median food intake of the vehicle treated animals. At the onset of the study, there was no significant difference in body weight between the three experimental groups (day 1 body weight: 342 ± 9 g, [vehicle] vs 365 ± 7 g, [H3 *ad libitum* fed] vs 352 ± 10 g [H3 pair-fed]). Twenty-four hour food intake in the H3 *ad libitum* fed group was significantly higher compared to vehicle at the beginning and end of the study [day 1: 23 ± 3 g (vehicle) vs 34 ± 4 g (H3), $p< 0.05$, and day 7: 33 ± 1 g (vehicle) vs 38 ± 1 g (H3), $p< 0.05$] (Figure 7A). IntraPVN injection of 180 pmol H3 stimulated feeding during the first hour following injection in both the early light phase and early dark phase on each study day. There was no attenuation in the acute orexigenic response in the early light phase on repeated administration up to 7 days [0-1 hour food intake: 0.3 ± 0.2 g (vehicle) and 2.3 ± 0.6 g (H3) (day 1) vs 0.7 ± 0.3 g (vehicle) and 3.0 ± 0.7 g (H3) (day 7), $p< 0.05$ vs vehicle] (Figure 7B). Trend analysis of cumulative food intake revealed that over the period of the study the H3-treated *ad libitum* fed group ate significantly more than vehicle-treated *ad libitum* fed controls [211.8 ± 7.1 g (vehicle) vs 261.6 ± 6.7 g (H3), $p< 0.05$ vs vehicle at the beginning of day 7] (Figure 8). There was also a trend towards greater cumulative body weight change in the H3-treated *ad libitum* fed group compared to vehicle-treated animals [8 ± 4 g (vehicle) vs 13 ± 4 g (H3)]. There was no difference in cumulative body weight change between the H3 pair-fed group compared to vehicle at day 7 [8 ± 4g (vehicle) vs 2 ± 2 g (H3 pair-fed)], but body weight change was significantly reduced in the H3 pair-fed compared to H3-treated *ad libitum* fed animals [2 ± 2 g (H3 pair-fed) vs 13 ± 4g (H3 *ad libitum* fed), $p <0.05$].

Example 12: Effects of 7 day repeated iPVN administration of relaxin-3 on fat mass, UCP-1 expression and plasma hormones

Following an iPVN injection of H3 (180 pmol) or vehicle twice a day for 7 days, there was no significant change in interscapular BAT weight [0.54 ± 0.03 g (vehicle) vs 0.61 ± 0.04 g (H3 *ad libitum* fed) vs 0.56 ± 0.04 g (H3 pair-fed)]. There was no difference

observed in interscapular BAT UCP-1 mRNA expression between the experimental groups [12.83 ± 1.80 (vehicle) vs 14.28 ± 2.36 (H3 *ad libitum* fed) vs 12.42 ± 2.65 (H3 pair-fed), units are arbitrary] (Table 2).

There was no significant difference in epididymal fat mass between the 3 groups [3.35 ± 0.27 g (vehicle) vs 4.05 ± 0.32 g (H3 *ad libitum* fed) vs 3.31 ± 0.23 g (H3 pair-fed)]. However, plasma leptin was significantly elevated in the H3 *ad libitum* fed group compared to vehicle, [2.24 ± 0.40 ng/ml (vehicle) vs 3.67 ± 0.55 ng/ml (H3 *ad libitum* fed) vs 2.05 ± 0.49 (H3 pair-fed), $p < 0.05$ H3 *ad libitum* fed vs vehicle] (Table 2).

Table 2: The effect of 7-day repeated iPVN administration of vehicle or H3 (180pmol) on epididymal fat pad weight (WAT), BAT weight, UCP-1 mRNA expression, plasma leptin, plasma TSH and free T3.

	Vehicle	**H3 (180 pmol)**	**H3 PF (180 pmol)**
WAT (g)	3.35 ± 0.27	4.05 ± 0.32	3.31 ± 0.23
Leptin (ng/ml)	2.24 ± 0.40	3.67 ±0.55 *	2.05 ± 0.49
BAT (g)	0.54 ± 0.03	0.61 ± 0.04	0.56 ± 0.04
UCP-1 mRNA (U)	12.83 ± 1.80	14.28 ± 2.36	12.42 ± 2.65
TSH (ng/ml)	3.93 ± 0.53	2.44 ± 0.30*	2.59 ± 0.36*
Free T3 (pg/ml)	1.24 ± 0.14	1.53 ± 0.14	1.76 ± 0.22

Vehicle – vehicle-treated, *ad libitum* fed; **H3** – H3-treated, *ad libitum* fed; **H3 PF** – H3-treated, pair-fed to median food intake of vehicle-treated animals. Data shown as mean ± SEM. * = $p < 0.05$ vs vehicle

A significant suppression of plasma TSH was observed in both the H3 *ad libitum* fed group and H3 pair-fed group when compared to vehicle-treated animals [3.93 ± 0.53 ng/ml (vehicle) vs 2.44 ± 0.30 ng/ml (H3 *ad libitum* fed), $p<0.05$, vs 2.59 ± 0.36 ng/ml (H3 pair-fed)] (Figure 9). There was no significant difference in plasma free T3 levels

between the groups [1.24 ± 0.14 pg/ml (vehicle) vs 1.53 ± 0.14 pg/ml (H3 *ad libitum* fed) vs 1.76 ± 0.22 pg/ml (H3 pair-fed)].

There were no significant differences in adrenal and testicular weight between the treatment groups and no differences in plasma prolactin, corticosterone, LH and GH plasma levels amongst the three groups (data not shown).

Example 13: Effect of relaxin-3 on in vitro release of hypothalamic neuropeptides

To examine the possible central mediators of the effects of H3 on plasma TSH, hypothalamic release of neuropeptides known to regulate thyroid function (TRH and SRIF) was determined in response to H3 relaxin *in vitro*. TRH was significantly reduced by 10 nM H3 (100 ± 7.5 % basal vs 77.2 ± 7.2 % basal release, $p < 0.05$) whilst SRIF release increased significantly in response to 10 nM H3 (100 ± 8.1 % basal vs 163.6 ± 21.1 % basal release, $p < 0.05$).

Claims

1. A method for identifying compounds which will be useful as agents for the control of appetite and/or food intake in a mammal, comprising contacting a candidate molecule with a GPCR135 and monitoring the interaction of the candidate molecule and the GPCR135.
2. A method for identifying compounds which will be useful in the treatment of obesity, comprising contacting a candidate molecule with GPCR135 and monitoring the interaction of the candidate molecule and the GPCR135.
3. A method for identifying compounds which will be useful as agents for the control of appetite and/or food intake, comprising ascertaining the binding affinity of a candidate molecule to GPCR135 receptor.
4. A method according to claim 3, which comprises ascertaining the GPCR135 agonist activity of the candidate molecule.
5. A method according to claim 4, which comprises determining whether the GPCR135 agonist activity of the candidate molecule is greater than or equal to the agonist activity of human relaxin-3 under essentially the same conditions.
6. A method according to claim 3, which comprises ascertaining the GPCR135 antagonist activity of the candidate molecule.
7. A method according to any one of claims 1 to 6, in which the GPCR135 is human GPCR135.
8. A method according to any one of claims 1 to 6, in which the GPCR135 is rat GPCR135.
9. A method according to any one of claims 1 to 6, in which the GPCR135 is mouse GPCR135.
10. A method according to any one of claims 1 to 6, in which the GPCR135 has at least 75% homology with human GPCR135.
11. A method according to claim 10, in which the GPCR135 has at least 95% homology with human GPCR135.
12. A compound identified by a method according to any one of the preceding claims.

13. A compound identified by a method according to any one of the preceding claims, for use in a method of treatment of the human or animal body.

14. Relaxin-3, for use in a method of treatment of the human or animal body for an eating disorder.

15. Relaxin-3, for use in a method of treatment of anorexia nervosa or cachexia.

16. A pharmaceutical composition comprising a compound according to claim 13 or a physiologically acceptable salt, solvate or derivative thereof, together with one or more pharmaceutically acceptable carriers.

17. A pharmaceutical composition according to claim 16, in which the compound is a GPCR135 antagonist and is present in a therapeutically effective amount for the treatment of obesity.

18. The use of a compound which is an antagonist of GPCR135, or a physiologically acceptable salt, solvate or derivative thereof, for the manufacture of a medicament for suppression of appetite and/or food uptake.

19. The use of a compound which is an agonist of GPCR135, or a physiologically acceptable salt, solvate or derivative thereof, for the manufacture of a medicament for stimulation of appetite and/or food uptake.

20. A method for the treatment of an eating disorder in a mammal, including a human, comprising administration of a compound which agonises GPCR135.

21. A method as claimed in claim 20 or a use as claimed in claim 19 in which the disorder is cachexia.

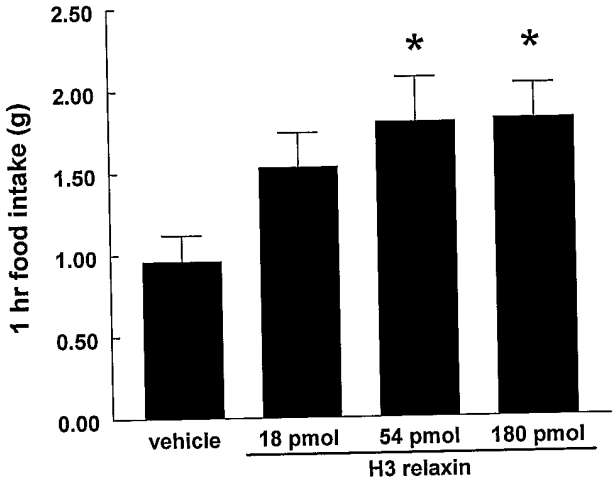
22. A method according to claim 20, a use as claimed in claim 19 or a method or a use as claimed in claim 21 in which the compound is relaxin-3.

23. A method for the treatment of obesity in a mammal, including a human, comprising administration of a compound which antagonises GPCR135.

24. A method for cosmetic weight loss in a mammal, the method comprising administering a composition comprising a GPCR135 antagonist to a mammal.

Figure 1

A


2.50
2.00
1.50
1.00
0.50
0.00
1 hr food intake (g)
*
*
vehicle
18 pmol
54 pmol
180 pmol
H3 relaxin

B

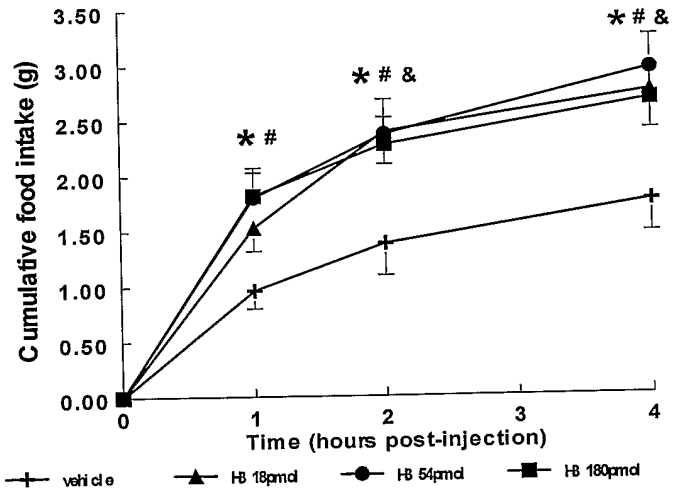
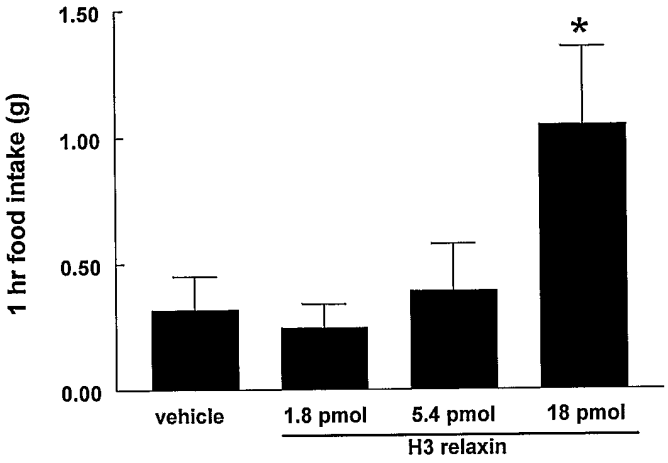

3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Cumulative food intake (g)
* #
* # &
* # &
0
1
2
3
4
Time (hours post-injection)
vehicle
H3 18pmol
H3 54pmol
H3 180pmol

Figure 2

A


1.50
1.00
0.50
0.00
1 hr food intake (g)
*
vehicle
1.8 pmol
5.4 pmol
18 pmol
H3 relaxin

B

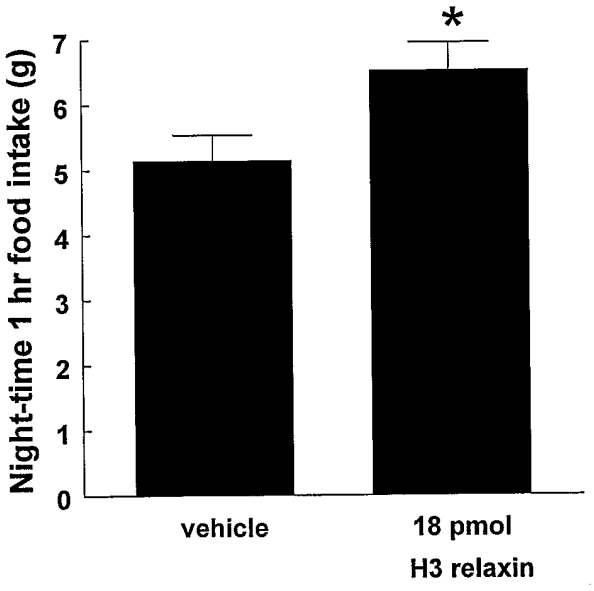

Night-time 1 hr food intake (g)
7
6
5
4
3
2
1
0
*
vehicle
18 pmol
H3 relaxin

Figure 3

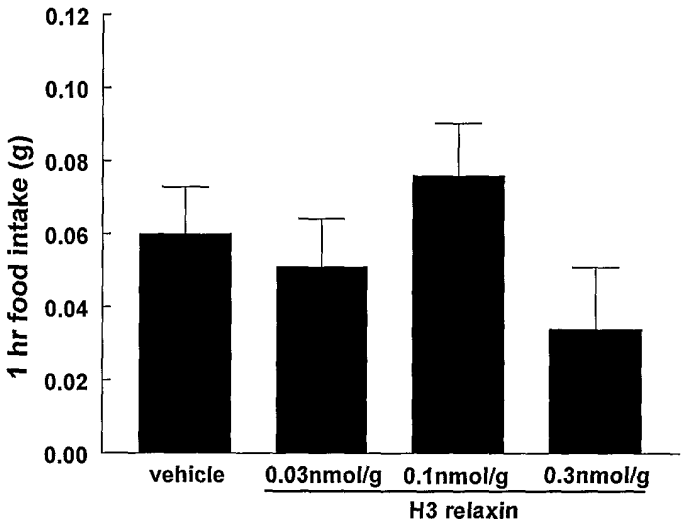

0.12
0.10
0.08
0.06
0.04
0.02
0.00
1 hr food intake (g)
vehicle
0.03nmol/g
0.1nmol/g
0.3nmol/g
H3 relaxin

Figure 4

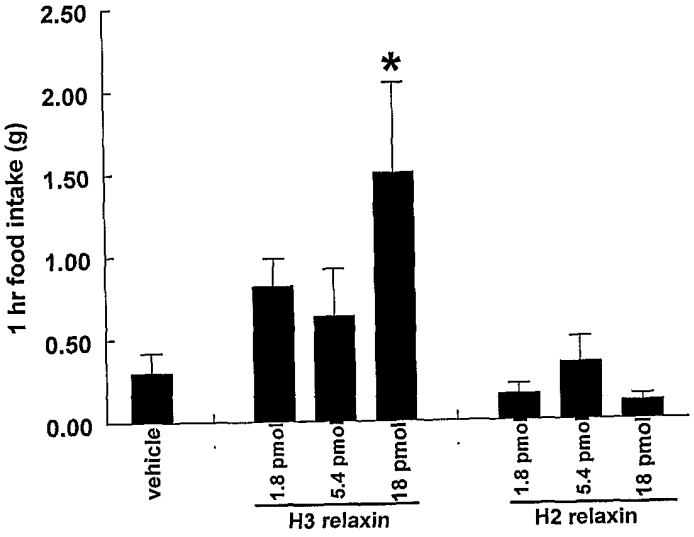

2.50
2.00
1.50
1.00
0.50
0.00
1 hr food intake (g)
*
vehicle
1.8 pmol
5.4 pmol
18 pmol
H3 relaxin
1.8 pmol
5.4 pmol
18 pmol
H2 relaxin

Figure 5

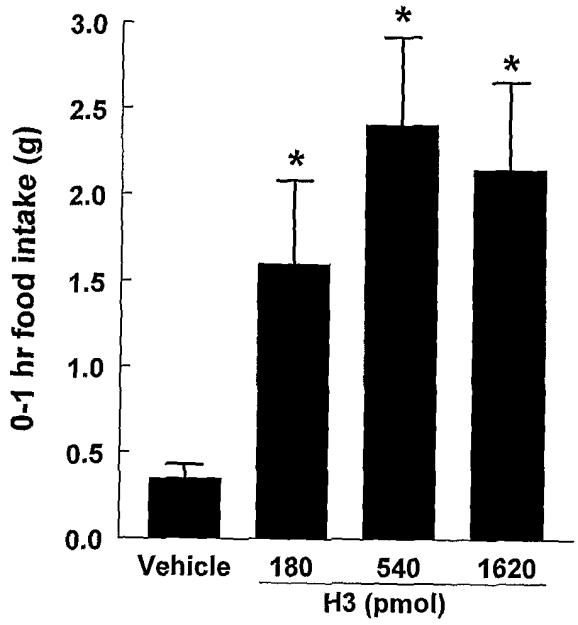

3.0
2.5
2.0
1.5
1.0
0.5
0.0
0-1 hr food intake (g)
*
*
*
Vehicle
180
540
1620
H3 (pmol)

Figure 6

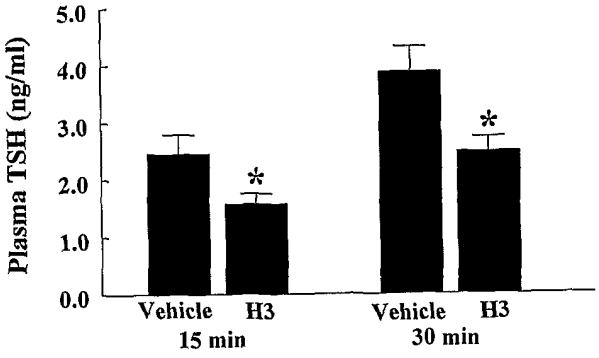

Plasma TSH (ng/ml)
5.0
4.0
3.0
2.0
1.0
0.0
*
*
Vehicle
H3
15 min
Vehicle
H3
30 min

Figure 7A

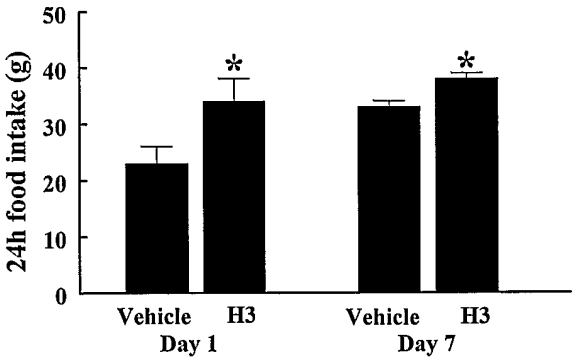


Figure 7B

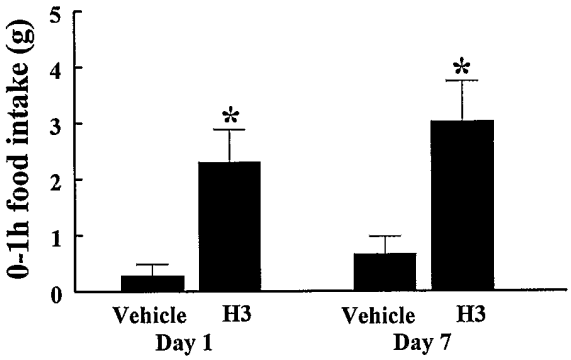


Figure 8

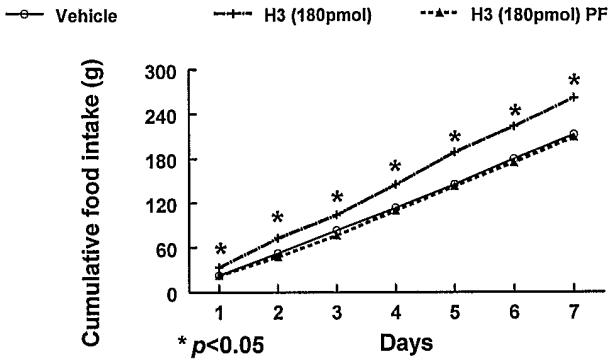
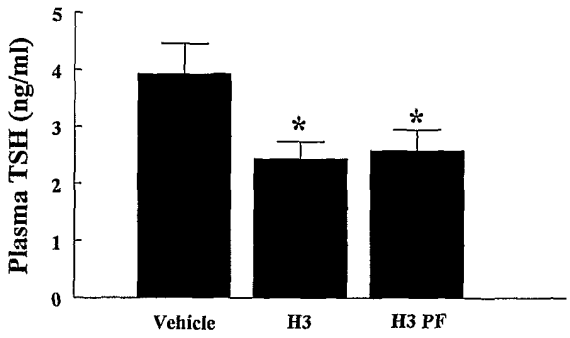

Vehicle
H3 (180pmol)
H3 (180pmol) PF
Cumulative food intake (g)
300
240
180
120
60
0
1
2
3
4
5
6
7
* p<0.05
Days

Figure 9



10/10

Figure 10

SEQUENCE LISTING

<110> Imperial Innovations Limited

<120> Improvements in or relating to appetite-influencing medicaments

<130> 10800 WO

<150> GB0504856.6

<151> 2005-03-09

<160> 3

<170> PatentIn version 3.1

<210> 1

<211> 469

<212> PRT

<213> Homo sapiens

<400> 1

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Glu Ala Ala Asn Thr Ser Gly Asn Ala Ser Leu Gln Leu Pro Asp Leu
35 40 45

Trp Trp Glu Leu Gly Leu Glu Leu Pro Asp Gly Ala Pro Pro Gly His
50 55 60

Pro Pro Gly Ser Gly Gly Ala Glu Ser Ala Asp Thr Glu Ala Arg Val
65 70 75 80

Arg Ile Leu Ile Ser Val Val Tyr Trp Val Val Cys Ala Leu Gly Leu
85 90 95

Ala Gly Asn Leu Leu Val Leu Tyr Leu Met Lys Ser Met Gln Gly Trp
100 105 110

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Arg Lys Ser Ser Ile Asn Leu Phe Val Thr Asn Leu Ala Leu Thr Asp
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Phe Gln Phe Val Leu Thr Leu Pro Phe Trp Ala Val Glu Asn Ala Leu
    130                 135                 140

Asp Phe Lys Trp Pro Phe Gly Lys Ala Met Cys Lys Ile Val Ser Met
145                 150                 155                 160

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

Ser Val Thr Arg Tyr His Ser Val Ala Ser Ala Leu Lys Ser His Arg
            180                 185                 190

Thr Arg Gly His Gly Arg Gly Asp Cys Cys Gly Arg Ser Leu Gly Asp
        195                 200                 205

Ser Cys Cys Phe Ser Ala Lys Ala Leu Cys Val Trp Ile Trp Ala Leu
    210                 215                 220

Ala Ala Leu Ala Ser Leu Pro Ser Ala Ile Phe Ser Thr Thr Val Lys
225                 230                 235                 240

Val Met Gly Glu Glu Leu Cys Leu Val Arg Phe Pro Asp Lys Leu Leu
                245                 250                 255

Gly Arg Asp Arg Gln Phe Trp Leu Gly Leu Tyr His Ser Gln Lys Val
            260                 265                 270

Leu Leu Gly Phe Val Leu Pro Leu Gly Ile Ile Ile Leu Cys Tyr Leu
        275                 280                 285

Leu Leu Val Arg Phe Ile Ala Asp Arg Arg Ala Ala Gly Thr Lys Gly
    290                 295                 300

Gly Ala Ala Val Ala Gly Gly Arg Pro Thr Gly Ala Ser Ala Arg Arg
305                 310                 315                 320

Leu Ser Lys Val Thr Lys Ser Val Thr Ile Val Val Leu Ser Phe Phe
                325                 330                 335

Leu Cys Trp Leu Pro Asn Gln Ala Leu Thr Thr Trp Ser Ile Leu Ile
            340                 345                 350

Lys Phe Asn Ala Val Pro Phe Ser Gln Glu Tyr Phe Leu Cys Gln Val
        355                 360                 365

Tyr Ala Phe Pro Val Ser Val Cys Leu Ala His Ser Asn Ser Cys Leu
    370                 375                 380
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Asn Pro Val Leu Tyr Cys Leu Val Arg Arg Glu Phe Arg Lys Ala Leu
385                 390                 395                 400

Lys Ser Leu Leu Trp Arg Ile Ala Ser Pro Ser Ile Thr Ser Met Arg
                405                 410                 415

Pro Phe Thr Ala Thr Thr Lys Pro Glu His Glu Asp Gln Gly Leu Gln
            420                 425                 430

Ala Pro Ala Pro Pro His Ala Ala Ala Glu Pro Asp Leu Leu Tyr Tyr
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Pro Pro Gly Val Val Val Tyr Ser Gly Gly Arg Tyr Asp Leu Leu Pro
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Ser Ser Ser Ala Tyr
465
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<212> PRT

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        35                  40                  45

Ser Leu Gln Leu Gln Asp Leu Trp Trp Glu Leu Gly Leu Glu Leu Pro
    50                  55                  60

Asp Gly Ala Ala Pro Gly His Pro Pro Gly Ser Gly Gly Ala Glu Ser
65                  70                  75                  80

Ala Asp Thr Glu Ala Arg Val Arg Ile Leu Ile Ser Ala Val Tyr Trp
                85                  90                  95

Val Val Cys Ala Leu Gly Leu Ala Gly Asn Leu Leu Val Leu Tyr Leu
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Met Lys Ser Lys Gln Gly Trp Arg Lys Ser Ser Ile Asn Leu Phe Val
        115                 120                 125
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Thr Asn Leu Ala Leu Thr Asp Phe Gln Phe Val Leu Thr Leu Pro Phe
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Trp Ala Val Glu Asn Ala Leu Asp Phe Lys Trp Pro Phe Gly Lys Ala
145                 150                 155                 160

Met Cys Lys Ile Val Ser Met Val Thr Ser Met Asn Met Tyr Ala Ser
                165                 170                 175

Val Phe Phe Leu Thr Ala Met Ser Val Ala Arg Tyr His Ser Val Ala
            180                 185                 190

Ser Ala Leu Lys Ser His Arg Thr Arg Gly His Gly Arg Gly Asp Cys
        195                 200                 205

Cys Gly Gln Ser Leu Gly Glu Ser Cys Cys Phe Ser Ala Lys Val Leu
    210                 215                 220

Cys Gly Leu Ile Trp Ala Ser Ala Ala Ile Ala Ser Leu Pro Asn Val
225                 230                 235                 240

Ile Phe Ser Thr Thr Ile Asn Val Leu Gly Glu Glu Leu Cys Leu Met
                245                 250                 255

His Phe Pro Asp Lys Leu Leu Gly Trp Asp Arg Gln Phe Trp Leu Gly
            260                 265                 270

Leu Tyr His Leu Gln Lys Val Leu Leu Gly Phe Leu Leu Pro Leu Ser
        275                 280                 285

Ile Ile Ser Leu Cys Tyr Leu Leu Leu Val Arg Phe Ile Ser Asp Arg
    290                 295                 300

Arg Val Val Gly Thr Thr Asp Gly Ala Thr Ala Pro Gly Gly Ser Leu
305                 310                 315                 320

Ser Thr Ala Gly Ala Arg Arg Arg Ser Lys Val Thr Lys Ser Val Thr
                325                 330                 335

Ile Val Val Leu Ser Phe Phe Leu Cys Trp Leu Pro Asn Gln Ala Leu
            340                 345                 350

Thr Thr Trp Ser Ile Leu Ile Lys Phe Asn Val Val Pro Phe Ser Gln
        355                 360                 365

Glu Tyr Phe Gln Cys Gln Val Tyr Ala Phe Pro Val Ser Val Cys Leu
    370                 375                 380

Ala His Ser Asn Ser Cys Leu Asn Pro Ile Leu Tyr Cys Leu Val Arg
385                 390                 395                 400
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Arg Glu Phe Arg Lys Ala Leu Lys Asn Leu Leu Trp Arg Ile Ala Ser
405 410 415

Pro Ser Leu Thr Ser Met Arg Pro Phe Thr Ala Thr Thr Lys Pro Glu
420 425 430

Pro Glu Asp His Gly Leu Gln Ala Leu Ala Pro Leu Asn Ala Thr Ala
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<211> 472

<212> PRT

<213> Mus musculus

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20 25 30

Glu Val Ala Asn Ala Ser Gly Asn Ala Ser Leu Gln Leu Gln Asp Leu
35 40 45

Trp Trp Glu Leu Gly Leu Glu Leu Pro Asp Gly Ala Ala Pro Gly His
50 55 60

Pro Pro Gly Gly Gly Gly Ala Glu Ser Thr Asp Thr Glu Ala Arg Val
65 70 75 80

Arg Ile Leu Ile Ser Ala Val Tyr Trp Val Val Cys Ala Leu Gly Leu
85 90 95

Ala Gly Asn Leu Leu Val Leu Tyr Leu Met Lys Ser Lys Gln Gly Trp
100 105 110

Arg Lys Ser Ser Ile Asn Leu Phe Val Thr Asn Leu Ala Leu Thr Asp
115 120 125

Phe Gln Phe Val Leu Thr Leu Pro Phe Trp Ala Val Glu Asn Ala Leu
130 135 140

```
Asp Phe Lys Trp Pro Phe Gly Lys Ala Met Cys Lys Ile Val Ser Met
145                 150                 155                 160

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

Ser Val Ala Arg Tyr His Ser Val Ala Ser Ala Leu Lys Ser His Arg
            180                 185                 190

Thr Arg Gly Arg Gly Arg Gly Asp Cys Cys Gly Gln Ser Leu Arg Glu
        195                 200                 205

Ser Cys Cys Phe Ser Ala Lys Val Leu Cys Gly Leu Ile Trp Ala Ser
    210                 215                 220

Ala Ala Leu Ala Ser Leu Pro Asn Ala Ile Phe Ser Thr Thr Ile Arg
225                 230                 235                 240

Val Leu Gly Glu Glu Leu Cys Leu Met His Phe Pro Asp Lys Leu Leu
                245                 250                 255

Gly Trp Asp Arg Gln Phe Trp Leu Gly Leu Tyr His Leu Gln Lys Val
            260                 265                 270

Leu Leu Gly Phe Leu Leu Pro Leu Ser Ile Ile Ser Leu Cys Tyr Leu
        275                 280                 285

Leu Leu Val Arg Phe Ile Ser Asp Arg Arg Val Val Gly Thr Thr Asp
    290                 295                 300

Ala Val Gly Ala Ala Ala Ala Pro Gly Gly Gly Leu Ser Thr Ala Ser
305                 310                 315                 320

Ala Arg Arg Arg Ser Lys Val Thr Lys Ser Val Thr Ile Val Val Leu
                325                 330                 335

Ser Phe Phe Leu Cys Trp Leu Pro Asn Gln Ala Leu Thr Thr Trp Ser
            340                 345                 350

Ile Leu Ile Lys Phe Asn Ala Val Pro Phe Ser Gln Glu Tyr Phe Gln
        355                 360                 365

Cys Gln Val Tyr Ala Phe Pro Val Ser Val Cys Leu Ala His Ser Asn
    370                 375                 380

Ser Cys Leu Asn Pro Ile Leu Tyr Cys Leu Val Arg Arg Glu Phe Arg
385                 390                 395                 400

Lys Ala Leu Lys Asn Leu Leu Trp Arg Ile Ala Ser Pro Ser Leu Thr
                405                 410                 415
```

```
Asn Met Arg Pro Phe Thr Ala Thr Thr Lys Pro Glu Pro Glu Asp His
            420                 425                 430

Gly Leu Gln Ala Leu Ala Pro Leu Asn Ala Ala Ala Glu Pro Asp Leu
        435                 440                 445

Ile Tyr Tyr Pro Pro Gly Val Val Val Tyr Ser Gly Gly Arg Tyr Asp
    450                 455                 460

Leu Leu Pro Ser Ser Ser Ala Tyr
465                 470
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INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2006/000823

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/50 G01N33/68 A61K38/22 A61K31/00 A61K38/00
C07K14/64

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2004/082598 A (JANSSEN PHARMACEUTICA N.V; CHEN, JINGCAI; KUEI, CHESTER; LIU, CHANGLU;) 30 September 2004 (2004-09-30) claims	
A	LIU C ET AL: "Identification of relaxin-3/INSL7 as an endogenous ligand for the orphan G-protein-coupled receptor GPCR135" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOCHEMICAL BIOLOGISTS, BIRMINGHAM,, US, vol. 278, no. 50, 12 December 2003 (2003-12-12), pages 50754-50764, XP002319829 ISSN: 0021-9258 the whole document	

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 June 2006	22/06/2006

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Vogt, T

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LIU C ET AL: "Identification of relaxin-3/INSL7 as a ligand for GPCR142" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOCHEMICAL BIOLOGISTS, BIRMINGHAM,, US, vol. 278, no. 50, 2003, pages 50765-50770, XP002987509 ISSN: 0021-9258	
P,X	MCGOWAN B M C ET AL: "Central relaxin-3 administration causes hyperphagia in male Wistar rats" ENDOCRINOLOGY, vol. 146, no. 8, August 2005 (2005-08), pages 3295-3300, XP002383675 ISSN: 0013-7227 Epub 21-04-2005 the whole document	1-24
P,X	WO 2005/040791 A (BAYER HEALTHCARE AG; GOLZ, STEFAN; BRUEGGEMEIER, ULF; GEERTS, ANDREAS) 6 May 2005 (2005-05-06) the whole document	1-24
P,X	WO 2005/075641 A (EISAI CO., LTD; HIDA, TAKAYUKI; HIROHASHI, TOMOKO; SAWAI, TORU; SEIKI,) 18 August 2005 (2005-08-18) abstract	1-24

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2006/000823

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 20-24
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 20-24 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 12,13,16-21,23,24
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.1

Although claims 20-24 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box II.2

Claims Nos.: 12,13,16-21,23,24

The present claims 12, 13,16-21,23 and 24 encompass compounds defined only by their desired function, contrary to the requirements of clarity of Article 6 PCT, because the result-to-be-achieved type of definition does not allow the scope of the claim to be ascertained.

The fact that any compound could be screened does not overcome this objection, as the skilled person would not have knowledge beforehand as to whether it would fall within the scope claimed, except for relaxin-3. Undue experimentation would be required to screen compounds randomly.

The search of claims 12, 13,16-21,23 and 24 was consequently restricted to relaxin-3.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2006/000823

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2004082598 A	30-09-2004	AU 2004222361 A1	30-09-2004
		CA 2517283 A1	30-09-2004
		EP 1599168 A2	30-11-2005
WO 2005040791 A	06-05-2005	NONE	
WO 2005075641 A	18-08-2005	NONE	