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Novel human G-protein-coupled receptors

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Abstract

G-protein-coupled receptors (GPCRs) are important mediators of signal transduction and targets for pharmacological therapeutics. Novel receptor–ligand systems have been discovered through the identification and analysis of orphan GPCRs (oGPCRs). Here we describe the discovery of seven novel human genes encoding oGPCRs. Each novel oGPCR gene was discovered using customized searches of the GenBank genomic databases with previously known GPCR-encoding sequences. The expressed genes can now be used in assays to determine endogenous and pharmacological ligands. *GPR133*, *GPR134*, *GPR135*, *GPR136*, and *GPR137* share identities with a prostate-specific odorant-like GPCR-encoding gene (*PSGR*). *GPR138* and *GPR139* share identities with an odorant-like gene derived from human erythroid cells. Transcripts encoding *GPR133*, *GPR134*, *GPR135*, *GPR136*, and *GPR137* were detected in various CNS tissues. The expression of odorant-like genes in non-olfactory tissues requires further clarification, which may be achieved through the search for endogenous cognate ligands for these and other oGPCRs.
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GPCRs are the largest family of cell surface mediators of signal transduction. They share high levels of homology due to their common topography consisting of seven transmembrane domains [1]. This feature has been useful in the discovery of novel receptors by means of homology-based strategies such as degenerate oligonucleotide PCR [2] and, more recently, through in silico database searches in GenBank (http://www.ncbi.nlm.nih.gov). The sequence of a receptor discovered in such a manner does not necessarily provide insights into the nature of its cognate ligand and therefore such receptors are termed as *orphan* GPCRs (oGPCRs) [3]. Orphan receptors have been used in ligand screening assays to discover novel endogenous receptor–ligand systems, such as apelin, melanin concentrating hormone, metastin, prolactin-releasing peptide, and ghrelin (recently reviewed in [4]). As of this writing, there are approximately 200 GPCRs for which ligands are known, and ~150 oGPCRs [5]. In addition, there are ~330 full-length human odorant-like GPCR genes known to date [6]. Specific odorant ligands have been found for only a few of the hundreds of candidate odorant receptors, and therefore the "odorant-like" classification for a novel receptor is defined by sequence homology. Odorant-like receptor genes have been discovered in the olfactory epithelium, as might be expected, but there is a growing number of genes which were discovered in tissues lacking any known olfactory function, suggesting that some odorant-like GPCRs are not involved in the chemosensory process [6]. As the sequencing of the human genome nears completion, we are continuing in our efforts to locate and identify the remaining GPCRs in the human genome. Most recently, we reported the discovery of 10 oGPCR-encoding genes and a pseudogene [7]. We now report the identification of seven additional oGPCR-encoding genes currently named *GPR133*, *GPR134*, *GPR135*, *GPR136*, *GPR137*, *GPR138*, and

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GPR139. *GPR133*, *GPR134*, *GPR135*, *GPR136*, and *GPR137*, as a group, most closely resemble a prostate-specific GPCR-encoding gene. *GPR138* and *GPR139* most closely resemble the odorant-like GPCRs.

Materials and methods

Database searching. We queried the non-redundant (nr) and high-throughput genomic sequences (HTGS) databases maintained by the National Center for Biotechnology Information (NCBI) with the amino acid sequences of various GPCRs using the TBLAST algorithm [8]. Returned genomic sequences having statistically significant scores were further examined. The conceptualized protein sequences encoded by these genomic sequences were used to query the nr database to determine whether these sequences encoded previously known GPCRs.

GPCR gene and cDNA cloning. To obtain DNA encoding novel GPCRs, human genomic DNA was amplified by PCR using the following oligonucleotides: *GPR133* (5′-CCC AGC ACC ACA TGT GTC AAC-3′, 5′-CAG ATA TTG TGT TCA TGA ACC-3′), *GPR134* (5′-CAT TAA TAG GCA AGA AGT CTC-3′, 5′-GAA GTT GGC TAG AAT GCT CTG-3′), *GPR135* (5′-CAC ATA TAA ATA GCC ATG CTC-3′, 5′-ATA GTA ATC ATC TAA TCT TCA-3′), *GPR136* (5′-TCA GCT TCT TCA TGA TGG TGG-3′, 5′-CAC TGA CAC CTA GGG CTC TGA-3′), *GPR137* (5′-CCT GGA TTT TGT ATG CAG AAG-3′, 5′-CAC TAA GGT GTC TCA CTT GCC-3′), *GPR138* (5′-CTA AAT GAT GGA CAA CCA CTC-3′, 5′-CAG AAC AGC TAA TCT TTC AGG-3′), and *GPR139* (5′-CTT TCT ATG TCT TTC ACT TCT C-3′, 5′-GGC TCA TCA TTG TGC CTT GC-3′). PCR conditions were as follows: denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 1 min. PCR products were purified, subcloned into the *Eco*RV site of pcDNA3 (Invitrogen, Carlsbad, CA), and sequenced.

Northern blot analyses. Human mRNA was extracted from various tissues as described previously [9]. Briefly, total RNA was extracted [10] and poly$(A)^+$ RNA was isolated using oligo(dT) cellulose spin columns (Pharmacia, Uppsala, Sweden). RNA was denatured and separated by electrophoresis on a 1% formaldehyde agarose gel, transferred onto nylon membrane, and immobilized by UV irradiation. The blots were hybridized with human GPCR-encoding ^{32}P-labeled DNA fragments, washed with 2× SSPE (3 M NaCl, 0.2 M NaH_2PO_4, and 0.02 M EDTA) and 0.1% SDS at 50 °C for 20 min, washed again with 0.1× SSPE and 0.1% SDS at 50 °C for 2 h, and exposed to X-ray film at −70 °C in the presence of an intensifying screen.

Results

Cloning of GPCR-encoding genes

A customized search of the HTGS database retrieved five candidate GPCR-encoding sequences clustered within a bacterial artificial chromosome (BAC) clone localized to human chromosome 11 (GenBank Accession No. AC090719). Primers were designed and used to PCR amplify the five DNA sequences. The PCR amplified products were cloned into pcDNA3 and

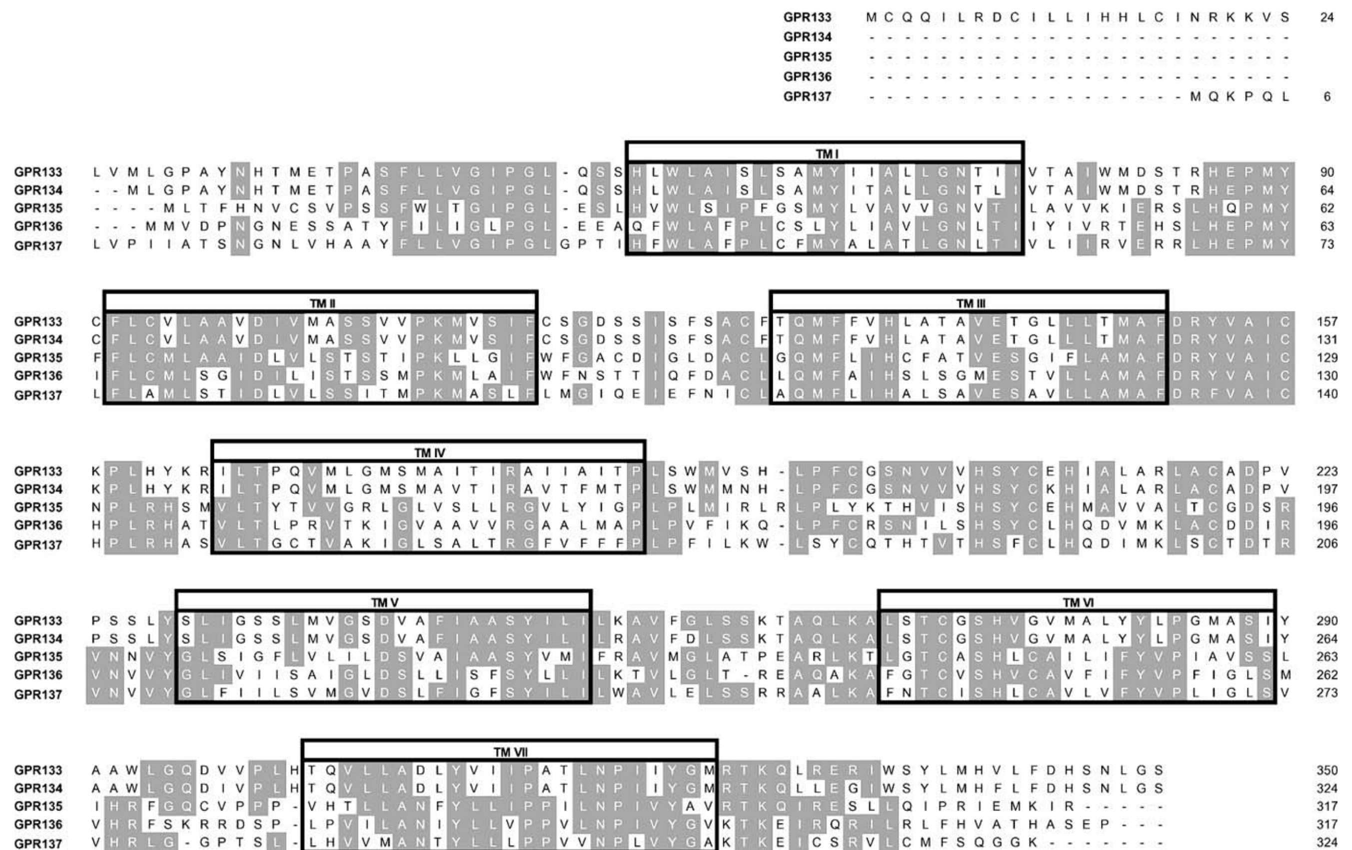

Fig. 1. Amino acid sequence alignment of GPR133, GPR134, GPR135, GPR136, and GPR137. Residues shared between at least three aligned receptors are shaded. Transmembrane domains (TMs) are indicated and amino acids are numbered on the right.

sequenced. These clones were named *GPR133*, *GPR134*, *GPR135*, *GPR136*, and *GPR137*, and they encoded proteins of 350, 324, 317, 318, and 325 amino acids in length, respectively (Fig. 1). *GPR133* and *GPR134* were 95% identical to each other, and *GPR136* and *GPR137* were 57% identical to each other. Overall, these five receptor genes were ∼40–50% identical to each other. These genes shared highest identities with a prostate-specific odorant-like orphan GPCR (*PSGR*) [11–13], which has been found to be overexpressed in prostate cancer [11,12]. GPR134 was the least similar receptor (40% homology to *PSGR* within TMs), while GPR136 was the most similar receptor (60% homology to *PSGR* within TMs).

A second customized search of the HTGS database using sequence fragments of various GPCRs yielded two candidate GPCR-encoding sequences located within a BAC clone localized to human chromosome 7 (GenBank Accession No. AC099652). The PCR amplified products (*GPR138* and *GPR139*) encoded proteins of 310 and 399 amino acids in length, respectively (Fig. 2). *GPR138* and *GPR139* most closely resemble the odorant-like GPCRs. *GPR138* and *GPR139* both share 42% homology within the TM regions with the SD_{olf} receptor, an OR derived from hematopoietic stem cells (GenBank Accession No. NP_277054).

Expression analyses

GPR133 mRNA transcripts were detected in human pituitary (1 kb) and putamen (two signals of 1.5 and 1.75 kb in size). No mRNA transcripts of *GPR133* were detected in the pons, hippocampus, thalamus, medulla, midbrain, hypothalamus, amygdala, and frontal cortex (Fig. 3).

GPR134 mRNA transcripts were detected in the frontal cortex, basal forebrain, midbrain, hippocampus, hypothalamus, and thalamus (one signal of 2.1 kb in size). *GPR134* transcripts were not detected in the caudate (Fig. 4).

GPR135 mRNAs were detected in the pituitary gland, with none observed in the frontal cortex, hippocampus, caudate, or nucleus accumbens. *GPR136* mRNA

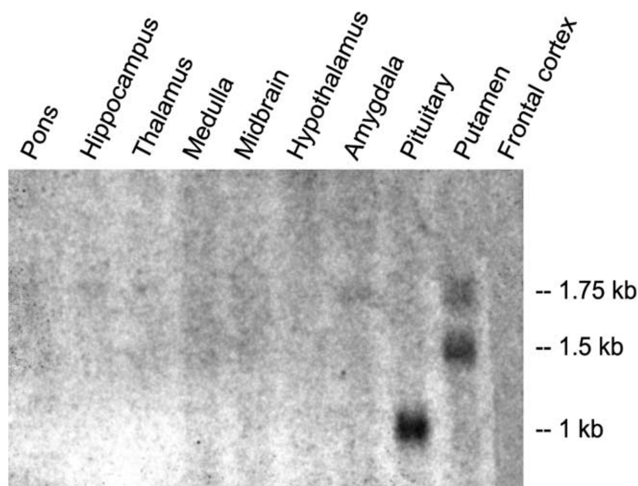

Fig. 3. Expression analysis of *GPR133*. Northern blot analysis of *GPR133* mRNA expression in various CNS tissues.

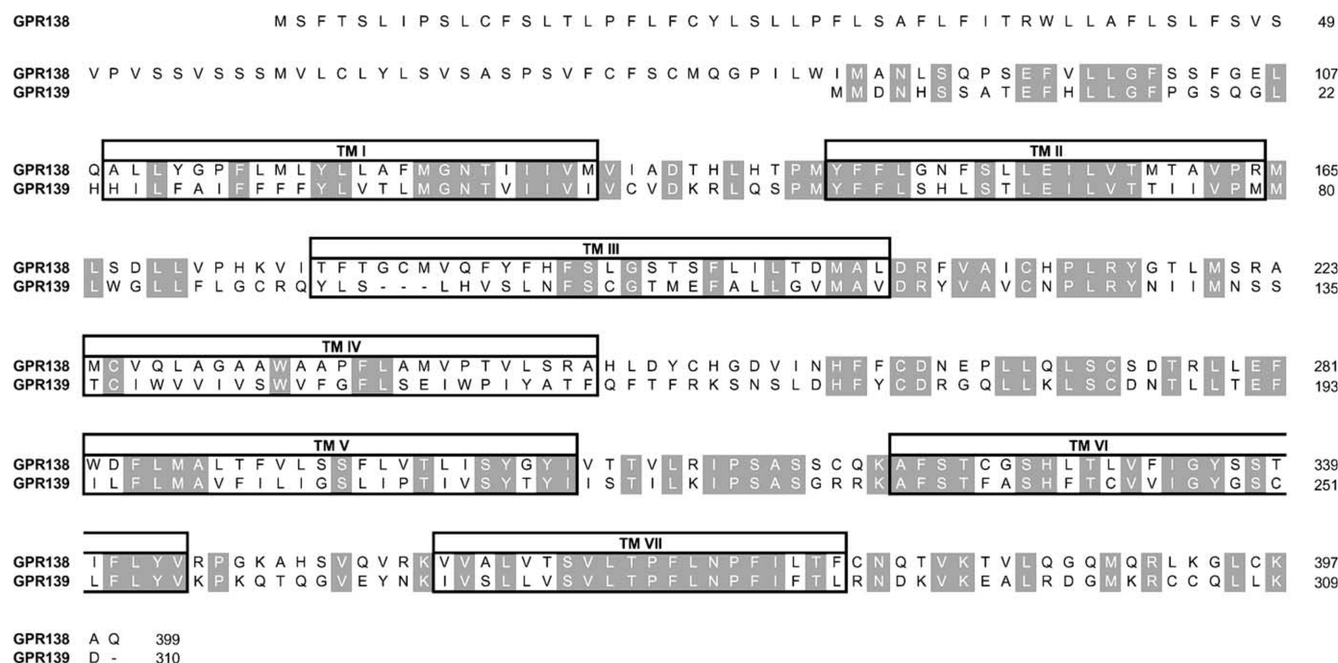

Fig. 2. Amino acid sequence alignment of GPR138 and GPR139. Residues shared between the aligned receptors are shaded. Transmembrane domains (TMs) are indicated and amino acids are numbered on the right.

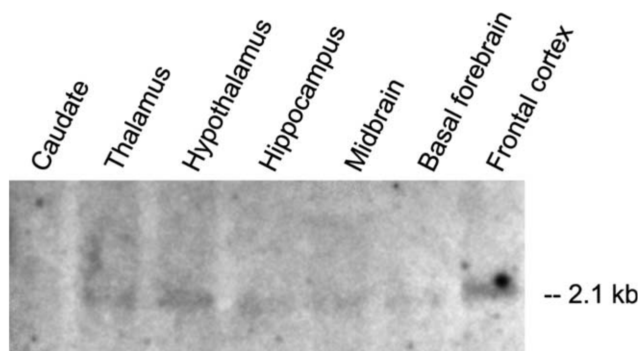

Fig. 4. Expression analysis of *GPR134*. Northern blot analysis of *GPR134* mRNA expression in various CNS tissues.

transcripts were detected in the frontal cortex, hypothalamus, midbrain, thalamus, hippocampus, basal forebrain, and caudate. No *GPR136* mRNA transcripts were detected in the liver. *GPR137* transcripts were detected in the hippocampus and not in the hypothalamus, midbrain, thalamus, pons, or basal forebrain (data not shown).

Discussion

In this report, we have described the discovery of seven novel human oGPCRs. The receptor genes are divided into two groups. *GPR133*, *GPR134*, *GPR135*, *GPR136*, and *GPR137* are closely related to each other, and their presence within the same human contig suggests a tight clustering of these genes. As a group, these receptor genes most closely resembled an orphan receptor (*PSGR*) which shows prostate-specific localization [11–13], as well as some expression in the olfactory zone and the medulla oblongata of the human brain [13]. Interestingly, *GPR133* and *GPR134*, while nearly identical (95%), are expressed in different CNS tissues. *GPR135*, *GPR136*, and *GPR137* also display differential expression in various tissues, suggesting that this family of receptors may have diverse roles in the CNS. Notably, *GPR136* has much wider distribution in the CNS than its congeners. *GPR138* and *GPR139* were most similar to an odorant-like receptor originally found in a hematopoietic cell line. The expression data for these and many other odorant-like receptors suggest that there exist endogenous ligands specific for these GPCRs, and therefore the search for ligands should not be restricted to odorant molecules.

As described in our recent review [5], there are currently ~350 human non-odorant GPCRs known to exist, over a third of which have no known cognate ligand. In addition, there are ~330 intact odorant-like genes, and approximately 600 odorant-like pseudogenes in human [6]. The efforts to identify and catalog all human GPCR-encoding genes are ongoing, and these efforts have resulted in the identification of entirely novel signaling systems such as apelin [14], melanin-concentrating hormone [15–19], metastin [20–22], prolactin-releasing peptide [23], orexin [24], and urotensin II [25–28]. These discoveries were made possible through the use of the orphan receptor in ligand screening assays and thus the identification of novel orphan GPCRs is an important first step in this process. In particular, the study of odorant-like receptors may lead to unexpected discoveries in signaling systems. Odorant-like receptors were first cloned in 1991 [29] and are now known to comprise the largest and most diverse subfamily of GPCRs [6]. Only one human odorant-like receptor has been assigned an olfactory agonist [30], while other odorant-like receptors have been implicated in the detection of sweet-tasting [31] and bitter-tasting [32] compounds. Most of the remaining odorant-like receptors have been classified as such due to sequence homology alone. It is unlikely that all ~330 human odorant-like receptors are solely involved in the detection of odors, since many are expressed in tissues with no known odor recognition function, such as human testes and erythroid cells. In addition, odorant-like receptors have been found in the brainstem of rat and mouse [6]. Therefore, the current classification of odorant receptors should be considered preliminary, until additional pharmacological and expression data can be gathered.

In conclusion, we have identified seven novel GPCR genes, named *GPR133*, *GPR134*, *GPR135*, *GPR136*, *GPR137*, *GPR138*, and *GPR139*. Transcripts for *GPR133*, *GPR134*, *GPR135*, *GPR136*, and *GPR137* were detected in various CNS tissues, which indicates that, although structurally homologous to odorant receptors, these may be activated by a novel class of brain signaling molecules. Given the high levels of identity shared by some of these genes, future efforts will likely discover common endogenous ligands for some, if not all, of the encoded receptors.

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References

[1] K.L. Pierce, R.T. Premont, R.J. Lefkowitz, Seven-transmembrane receptors, Nat. Rev. Mol. Cell Biol. 3 (9) (2002) 639–650.
[2] A. Marchese, S.R. George, B.F. O'Dowd, Cloning of G-protein-coupled receptor genes: the use of homology screening and the polymerase chain reaction, in: K.R. Lynch (Ed.), Identification and Expression of G-Protein-Coupled Receptors, Wiley-Liss, New York, 1998, pp. 1–28.
[3] A. Marchese, S.R. George, L.F. Kolakowski Jr., K.R. Lynch, B.F. O'Dowd, Novel GPCRs and their endogenous ligands:

expanding the boundaries of physiology and pharmacology, Trends Pharmacol. Sci. 20 (9) (1999) 370–375.

[4] D.K. Lee, S.R. George, B.F. O'Dowd, Novel G-protein-coupled receptor genes expressed in the brain: continued discovery of important therapeutic targets, Expert Opin. Ther. Targets 6 (2) (2002) 185–202.

[5] W.J. Bailey, W.B. Vanti, S.R. George, R. Blevins, S. Swaminathan, J.A. Bonini, K.E. Smith, R.L. Weinshank, B.F. O'Dowd, Patent status of the therapeutically important G-protein-coupled receptors, Expert Opin. Ther. Patents 11 (12) (2001) 1861–1887.

[6] P. Mombaerts, The human repertoire of odorant receptor genes and pseudogenes, Annu. Rev. Genom. Hum. Genet. 2 (2001) 493–510.

[7] D.K. Lee, T. Nguyen, K.R. Lynch, R. Cheng, W.B. Vanti, O. Arkhitko, T. Lewis, J.F. Evans, S.R. George, B.F. O'Dowd, Discovery and mapping of ten novel G-protein-coupled receptor genes, Gene 275 (1) (2001) 83–91.

[8] S.F. Altschul, T.L. Madden, A.A. Schaffer, J. Zhang, W. Miller, D.J. Lipman, Gapped BLAST and PSI-BLAST: a new generation of protein database search programs, Nucleic Acids Res. 25 (1997) 3389–3402.

[9] A. Marchese, J.M. Docherty, T. Nguyen, M. Heiber, R. Cheng, H.H. Heng, L.C. Tsui, X. Shi, S.R. George, B.F. O'Dowd, Cloning of human genes encoding novel G-protein-coupled receptors, Genomics 23 (1994) 609–618.

[10] P. Chomczynski, N. Sacchi, Single-step method of RNA isolation by acid guanidium thiocyanate–phenol–chloroform extraction, Anal. Biochem. 162 (1987) 156–159.

[11] L.L. Xu, B.G. Stackhouse, K. Florence, W. Zhang, N. Shanmugan, I.A. Sesterhenn, Z. Zou, V. Srikantan, M. Augustus, V. Roschke, K. Carter, D.G. McLeod, J.W. Moul, D. Soppett, S. Srivastava, PSGR, a novel prostate-specific gene with homology to a G-protein-coupled receptor, is overexpressed in prostate cancer, Cancer Res. 60 (23) (2000) 6568–6572.

[12] C. Xia, W. Ma, F. Wang, S.B. Hua, M. Liu, Identification of a prostate-specific G-protein-coupled receptor in prostate cancer, Oncogene 20 (41) (2001) 5903–5907.

[13] T.T. Yuan, P. Toy, J.A. McClary, R.J. Lin, N.G. Miyamoto, P.J. Kretschmer, Cloning and genetic characterization of an evolutionarily conserved human olfactory receptor that is differentially expressed across species, Gene 278 (1–2) (2001) 41–51.

[14] K. Tatemoto, M. Hosoya, Y. Habata, R. Fujii, T. Kakegawa, M.X. Zou, Y. Kawamata, S. Fukusumi, S. Hinuma, C. Kitada, T. Kurokawa, H. Onda, M. Fujino, Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor, Biochem. Biophys. Res. Commun. 251 (1998) 471–476.

[15] D. Bachner, H. Kreienkamp, C. Weise, F. Buck, D. Richter, Identification of melanin concentrating hormone (MCH) as the natural ligand for the orphan somatostatin-like receptor 1 (SLC-1), FEBS Lett. 457 (1999) 522–524.

[16] J. Chambers, R.S. Ames, D. Bergsma, A. Muir, L.R. Fitzgerald, G. Hervieu, G.M. Dytko, J.J. Foley, J. Martin, W.S. Liu, J. Park, C. Ellis, S. Ganguly, S. Konchar, J. Cluderay, R. Leslie, S. Wilson, H.M. Sarau, Melanin-concentrating hormone is the cognate ligand for the orphan G-protein-coupled receptor SLC-1, Nature 400 (1999) 261–265.

[17] P.M. Lembo, E. Grazzini, J. Cao, D.A. Hubatsch, M. Pelletier, C. Hoffert, S. St-Onge, C. Pou, J. Labrecque, T. Groblewski, D. O'Donnell, K. Payza, S. Ahmad, P. Walker, The receptor for the orexigenic peptide melanin-concentrating hormone is a G-protein-coupled receptor, Nat. Cell Biol. 1 (1999) 267–271.

[18] Y. Saito, H.P. Nothacker, Z. Wang, S.H.S. Lin, F. Leslie, O. Civelli, Molecular characterization of the melanin-concentrating hormone receptor, Nature 400 (1999) 265–269.

[19] Y. Shimomura, M. Mori, T. Sugo, Y. Ishibashi, M. Abe, T. Kurokawa, H. Onda, O. Nishimura, Y. Sumino, M. Fujino, Isolation and identification of melanin-concentrating hormone as the endogenous ligand of the SLC-1 receptor, Biochem. Biophys. Res. Commun. 261 (1999) 622–626.

[20] A.I. Muir, L. Chamberlain, N.A. Elshourbagy, D. Michalovich, D.J. Moore, A. Calamari, P.G. Szekeres, H.M. Sarau, J.K. Chambers, P. Murdock, K. Steplewski, U. Shabon, J.E. Miller, S.E. Middleton, J.G. Darker, C.G. Larminie, S. Wilson, D.J. Bergsma, P. Emson, R. Faull, K.L. Philpott, D.C. Harrison, AXOR12, a novel human G protein-coupled receptor, activated by the peptide KiSS-1, J. Biol. Chem. 276 (31) (2001) 28969–28975.

[21] M. Kotani, M. Detheux, A. Vandenbogaerde, D. Communi, J.M. Vanderwinden, E. Le Poul, S. Brezillon, R. Tyldesley, N. Suarez-Huerta, F. Vandeput, C. Blanpain, S.N. Schiffmann, G. Vassart, M. Parmentier, The metastasis suppressor gene KiSS-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54, J. Biol. Chem. 276 (37) (2001) 34631–34636.

[22] T. Ohtaki, Y. Shintani, S. Honda, H. Matsumoto, A. Hori, K. Kanehashi, Y. Terao, S. Kumano, Y. Takatsu, Y. Masuda, Y. Ishibashi, T. Watanabe, M. Asada, T. Yamada, M. Suenaga, C. Kitada, S. Usuki, T. Kurokawa, H. Onda, O. Nishimura, M. Fujino, Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor, Nature 411 (6837) (2001) 613–617.

[23] S. Hinuma, Y. Habata, R. Fujii, Y. Kawamata, M. Hosoya, S. Fukusumi, C. Kitada, Y. Masuo, T. Asano, H. Matsumoto, M. Sekiguchi, T. Kurokawa, O. Nishimura, H. Onda, M. Fujino, A prolactin-releasing peptide in the brain, Nature 393 (1998) 272–276.

[24] T. Sakurai et al., Orexins and orexin receptors: a family of hypothalamic neuropeptides and G-protein-coupled receptors that regulate feeding behavior, Cell 92 (1998) 573–585.

[25] R.S. Ames, H.M. Sarau, J.K. Chambers, R.N. Willette, N.V. Aiyar, A.M. Romanic, C.S. Louden, J.J. Foley, C.F. Sauermelch, R.W. Coatney, Z. Ao, J. Disa, S.D. Holmes, J.M. Stadel, J.D. Martin, W.S. Liu, G.I. glover, S. Wilson, D.E. McNulty, C.E. Ellis, et al., Human urotensin-II is a potent vasoconstrictor and agonist for the orphan receptor GPR14, Nature 401 (1999) 282–286.

[26] Q. Liu, S.S. Pong, Z. Zeng, Q. Zhang, A.D. Howard, D.L. Williams Jr., M. Davidoff, R. Wang, C.P. Austin, T.P. McDonald, C. Bai, S.R. George, J.F. Evans, C.T. Caskey, Identification of urotensin II as the endogenous ligand for the orphan G-protein-coupled receptor GPR14, Biochem. Biophys. Res. Commun. 266 (1999) 174–178.

[27] M. Mori, T. Sugo, M. Abe, Y. Shimomura, M. Kurihara, C. Kitada, K. Kikuchi, Y. Shintani, T. Kurokawa, H. Onda, O. Nishimura, M. Fujino, Urotensin II is the endogenous ligand of a G-protein-coupled orphan receptor, SENR (GPR14), Biochem. Biophys. Res. Commun. 265 (1999) 123–129.

[28] H.P. Nothacker, Z. Wang, Z.M. McNeill, Y. Saito, S. Merten, B.F. O'Dowd, S.P. Duckles, O. Civelli, Identification of the natural ligand of an orphan G-protein-coupled receptor involved in the regulation of vasoconstriction, Nat. Cell Biol. 1 (1999) 383–385.

[29] L. Buck, R. Axel, A novel multigene family may encode odorant receptors: a molecular basis for odor recognition, Cell 65 (1991) 175–187.

[30] C.H. Wetzel, M. Oles, C. Wellerdieck, M. Kuczkowiak, G. Gisselmann, H. Hatt, Specificity and sensitivity of a human olfactory receptor functionally expressed in human embryonic kidney 293 cells and *Xenopus laevis* oocytes, J. Neurosci. 19 (1999) 7426–7433.

[31] G. Nelson, M.A. Hoon, J. Chandrashekar, Y. Zhang, N.J. Ryba, C.S. Zuker, Mammalian sweet taste receptors, Cell 106 (2001) 381–390.

[32] J. Chandrashekar, K.L. Mueller, M.A. Hoon, E. Adler, L. Feng, W. Guo, C.S. Zuker, N.J. Ryba, T2Rs function as bitter taste receptors, Cell 100 (2000) 703–711.