The Metastasis Suppressor Gene KiSS-1 Encodes Kisspeptins, the Natural Ligands of the Orphan G Protein-coupled Receptor GPR54*

Masato Kotani‡‡, Michel Detheux†, Ann Vandenbergaaerdt‡, David Communi‡, Jean-Marie Vanderwinden‡‡‡, Emmanuel Le Poult†, Stéphane Brézillon‡, Richard Tyldesley‡‡, Nathalie Suarez-Huerta‡, Fabrice Vandeven‡, Cédric Blanpain‡‡‡, Serge N. Schiffmann**, Gilbert Vassart‡‡, and Marc Parmentier‡‡‡

From the Institute de Recherche Interdisciplinaire de Biologie Humaine et Nucléaire (I.R.I.B.H.N.), ‡‡‡Laboratoire de Neurophysiologie, and **Service de Génétique Médicale, Université Libre de Bruxelles, Campus Erasme, Route de Lennik 808, B-1070, Brussels, Belgium, †Euroscreen S.A., Route de Lennik 802, B-1070, Brussels, Belgium and ‡‡‡Micromass Ltd., Floats Rd., Wythenshawe, Manchester M23 9LZ, United Kingdom

Received for publication, May 28, 2001, and in revised form, July 11, 2001. Published, JBC Papers in Press, July 16, 2001, DOI 10.1074/jbc.M104847200

Natural peptides displaying agonist activity on the orphan G protein-coupled receptor GPR54 were isolated from human placenta. These 54-, 14-, and 13-amino acid peptides, with a common RF-amide C terminus, derive from the product of KiSS-1, a metastasis suppressor gene for melanoma cells, and were therefore designated kisspeptins. They bound with low nanomolar affinities to rat and human GPR54 expressed in Chinese hamster ovary K1 cells and stimulated IP2 hydrolysis, Ca2⁺ mobilization, arachidonic acid release, ERK1/2 and p38 MAP kinase phosphorylation, and stress fiber formation but inhibited cell proliferation. Human GPR54 was highly expressed in placenta, pituitary, pancreas, and spinal cord, suggesting a role in the regulation of endocrine function. Stimulation of oxytocin secretion after kisspeptin administration to rats confirmed this hypothesis.

G protein-coupled receptors constitute the largest family of membrane receptors (1). Over the last decade, a growing number of orphan G protein-coupled receptors have been made available by various cloning procedures such as PCR amplification using degenerate oligonucleotides and systematic sequencing of cDNA libraries and genomes. In human, in addition to about 160 characterized receptors, an equal number of genes encode proteins obviously belonging to this family of receptors, but their ligands and functions remain to be determined. These orphan receptors potentially constitute elements of fundamental communication pathways in various systems. Some of these orphan receptors have been used as tools to uncover these signaling pathways and have led to the validation of novel drug targets (2). Noicetin was the first of these novel ligands, purified from tissue extracts with the help of bioassays based on the expression of orphan receptors (3, 4). Other recent examples of new molecules identified as ligands of orphan receptors include orexins, involved in the control of feeding and sleep (5, 6), prolactin-releasing peptide (7), ghrelin (8), and apelin (9).

GPR54 is an orphan G protein-coupled receptor, originally cloned from rat brain and sharing 45% identity with galanine receptors (10). It is widely expressed in the rat central nervous system, including the hypothalamus, midbrain,pons, medulla, hippocampus, and amygdala (10). No natural or surrogate agonists of GPR54 have so far been identified, and its physiological functions are therefore unknown. The gene encoding human GPR54 is present in a bacterial artificial chromosome mapped to chromosome 2 (GenBank accession number AC023583).

In this work, we have tested tissue extracts on the basis of the distribution of GPR54 in human tissues. A biological activity was found in a placenta extract, and active peptides were purified and identified by mass spectrometry. They were found to derive from the previously described metastasis suppressor gene KiSS-1. The pharmacology of the peptides, signaling properties of the receptor, and in vivo function of this new system in vivo were investigated.

EXPERIMENTAL PROCEDURES

Tissue Distribution of GPR54 Expression in Human Tissues—RT-PCR experiments were carried out as described previously (11). Seventy-five ng of poly(A)⁺ RNA from various human tissues (CLONTECH) were tested using sense (5'TGTACAACTCTGTCGCGGTCG-3') and antisense (5'CCACCTGTCCTGGTTCTG-3') primers for human GPR54. Amplification of aldolase transcripts was performed in parallel as control.

Cloning and Expression of Rat and Human GPR54—The coding sequence of rat (GenBank accession number AF115516) and human (GenBank accession number AY029541) GPR54 was amplified by PCR from rat or human hypothalamus cDNA using rat sense (5'ATCGGAATTCACATGGCCGGCCAGGGCGAGCTG-3') and antisense (5'ACATCTTACATGAGGTCGGGTCGTTGTC-3') and human sense (5'ATCCGAAATTCACATGGCCGGCCAGGGCGAGCTG-3') and antisense (5'ATCATCTTACGTCAAGAAATGCGGTCGTTGTC-3') primers. Amplified DNA fragments were subcloned into the pEFN3 biocistic vector (12) and sequenced. These plasmids were transfected into CHO-K1 cells expressing Gα16 and a mitochondria-targeted form of apo-aequorin. Clonal cell lines were established, and

* This work was supported by the Actions de Recherche Concertées of the Communauté Française de Belgique, the Center de Recherche Inter-universitaire en Vaccinologie, the Belgian program on Interuniversity Poles of attraction initiated by the Belgian State, Prime Minister's Office, Science Policy Programming, the BIOMED, and Cell Factory program of the European Community (Grants BHR-CT98-2343 and QLK3-2000-00227), the Fonds de la Recherche Scientifique Médicale de Belgium, and the Fondation Médecine Reine Elisabeth (to M. P.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBankTM/EBI Data Bank with accession number(s) AY029541.

‡ Recipient of a fellowship of the Francqui Foundation.
‡‡ Aspirant of the Belgian Fonds National de la Recherche Scientifique.
†† To whom correspondence should be addressed. Tel.: 32-2-555-4172; Fax: 32-2-555-4655; E-mail: mparment@ulb.ac.be.

1 The abbreviations used are: PCR, polymerase chain reaction; RT, reverse transcription; CHO, Chinese hamster ovary; FCS, fetal calf serum; ERK, extracellular signal-regulated kinase; MAP, mitogen-activated protein; HPLC, high performance liquid chromatography.
functional responses were analyzed using a bioassay based on the luminescence of aequorin as a result of intracellular calcium release, as described previously (12). Briefly, cells (25,000 cells in 50 μl) were added to 50-μl samples in 96-well plates, and the luminescence was recorded for 30 s (PerkinElmer Life Sciences luminometer).

**Purification of Biological Activities—**Human placenta (1200 g) was homogenized in 6 liters of ice-cold methanol/water/acetic acid (90:9:1). The homogenate was centrifuged at 10,000 × g for 30 min at 4 °C, methanol was evaporated (Rotavapor), and lipids were extracted three times with dichloromethane. After trifluoroacetic acid (0.1%) addition, the extract was filtered (0.45 μm of Millipak 20 filter, Millipore), applied to a SepPak C18 column (40 × 300 mm, 15-μm beads, 300-Å pore size, Waters) and eluted with 80% acetonitrile, 0.1% trifluoroacetic acid at a flow rate of 100 ml/min. The eluate was loaded on the same column, a linear 0–80% acetonitrile gradient in 0.1% trifluoroacetic acid was applied over 80 min at 100 ml/min, and 50-ml fractions were collected. Fractions were tested on rat GPR54 using the aequorin-based assay. Active fractions were fractionated separately by two steps. First, a Symmetry 300 C18 column (4.6 × 250 mm, 5 μm, 300-Å, Waters) was eluted with a 0–27% acetonitrile gradient in 82 min, 27% acetonitrile for 30 min, and 27–50% gradient in 69 min at a flow rate of 1 ml/min. Second, a Vydc C18 column (1.0 × 250 mm, 5 μm, 300-Å, Vydc) was eluted with 20–35% methanol gradient in 1% trifluoroacetic acid over 15 min, 35–45% methanol gradient over 30 min, 45% methanol for 30 min, and 100% methanol gradient over 55 min at a flow rate of 50 μl/min. Fractions of 50 μl were collected. The active fractions were dried and analyzed by a prototype tandem matrix-assisted laser desorption ionization quadrupole time of flight mass spectrometer (Micromass).

**Binding Assays—**CHO-K1 cells (5 × 10⁵) were incubated with 125I-kisspeptin-10 (60,000 cpm, 2,200 Ci/mmol) as tracer and various test peptides as competitors in 300 μl of assay buffer as described in Langmead et al. (13) at 25 °C for 1 h, filtered through Whatman 934-AH filters preincubated with 0.5% polyethyleneimine, and washed with ice-cold buffer. Bound radioactivity was counted in a COBRA II counter (Packard Instrument Co.).

**Intracellular Calcium Release Assay—**For intracellular Ca²⁺ assays, cells pretreated or not with 100 ng/ml pertussis toxin for 15 h were incubated in Hank’s balanced salt medium containing 0.1% bovine serum albumin or vehicle alone as controls, modified Eagle’s medium/F-12 containing 0.1% bovine serum albumin and 2.8 μg/ml Fura-2 (Molecular Probes) at 37 °C for 45 min as described (11). Cells were used at a density of 10⁶/ml, and intracellular Ca²⁺ were measured using a luminescence spectrophotometer LS50B (PerkinElmer Life Sciences).

**Phosphoinositide Depletion Assay—**CHO-K1 cells were cultured overnight in DMEM containing 5% FCS and 1 μCi/ml myo-[3H]inositol (Amersham Pharmacia Biotech) detached with phosphate-buffered saline–EDTA, washed, and incubated for 20 min at 37 °C with kisspeptin-10 in 500 μl of 25 mM Tris-HCl, pH 7.4, 125 mM NaCl, 5.4 mM KCl, 0.8 mM MgCl₂, 1.8 mM CaCl₂, 15 mM glucose, and 10 mM LiCl. Incubation was terminated by adding 500 μl of buffer containing 88% methanol and 12% H₂O, and cells were filtered through Whatman GF/F filters and washed with ice-cold buffer. Bound radioactivity was measured in a TRI-CARB 2100TR counter (Packard Instrument Co.).

**Arachidonic Acid Release Assay—**Recombinant CHO-K1 cells (2.5 × 10⁶ cells/well in 6-well plates) were cultured overnight with 0.1 μCi/ml [3H]arachidonic acid (Amersham Pharmacia Biotech), washed, and incubated for 60 min at 37 °C with peptides in Dulbecco’s modified Eagle’s medium/F-12 containing 0.1% bovine serum albumin or vehicle alone as described (11). The radioactivity in the supernatant was measured in a TRI-CARB 2100TR counter (Packard Instrument Co.).

**ERK1/2, p38, and Stress-Activated Protein Kinase/c-Jun NH₂-terminal Kinase Activation Assays—**Recombinant CHO-K1 cells were cultured overnight in FCS-free medium, incubated with agonists or vehicle for various times, lysed in a buffer containing 50 mM Tris-HCl, pH 8.5, 8.7% glycerol, 0.1 mM diethiothreitol, 2% SDS, and heated to 100 °C for 5 min. Approximately 20 μg of proteins for ERK1/2 and 50 μg proteins for p38 and stress-activated protein kinase/c-Jun NH₂-terminal kinase were electrophoresed on 10% polyacrylamide gels and blotted to nylon membranes (PolyScreen, PerkinElmer Life Sciences). Phosphorylated ERK1/2, p38, and stress-activated protein kinase/c-Jun NH₂-terminal kinase were detected, respectively, with anti-phospho-p44/42 mitogen-activated protein kinase (MAP) E10 monoclonal antibody, anti-phospho-p38 MAP kinase polyclonal antibody, and anti-phospho-c-Jun NH₂-terminal kinase monoclonal antibody (Cell Signaling Technology) and an ECL detection reagent (Amersham Pharmacia Biotech).

**Cell Proliferation Assay—**For assaying proliferation, CHO-K1 cells (5 × 10⁵ cells/10-cm diameter dish) were seeded in 10% FCS medium and incubated with or without 1 μM kisspeptin-10 for 24, 48, and 72 h.

**RESULTS**

Distribution of Human GPR54—To identify potential sources of endogenous ligands, we tested the expression of GPR54 by RT-PCR in a set of human tissues. As shown in Fig. 1, GPR54 transcripts were particularly abundant in placenta, pituitary, spinal cord, and pancreas, whereas it was found at lower levels in other tissues, including various brain regions, stomach, small intestine, thymus, spleen, lung, testis, kidney, and fetal liver.

**Purification of Natural Ligands of GPR54—**CHO-K1 cell lines expressing rat GPR54 (Fig. 2) together with Gᵢₒ and apo-aequorin were used in a functional assay based on the luminescence of aequorin to identify natural ligands of the receptor. Extracts from various organs, including brain, placenta, intestine, stomach, lung, testis, and kidney were fractionated by HPLC, and individual fractions were tested in the aequorin-based assay using untransfected CHO-K1 cells and cell lines expressing other receptors as negative controls. Two peaks of biological activity specific for rat GPR54 were identified in fractions of a human placenta extract. These activities were purified through a number of HPLC columns (Fig. 3), and the active fractions resulting from the last step were analyzed by mass spectrometry. Sequencing and comparison of the corresponding masses with the protein and nucleotide data bases resulted in the identification of one peak in one of short overlapping peptides of 13 and 14 amino acids, terminating by a common C-terminal asparagine (Fig. 4). In the other peak, the same peptides were associated with a longer form of 54 amino acids with the same C terminus. All three peptides were predicted to derive from a previously identified protein named Kiss-1 (16). The three peptides were therefore designated as kisspeptin-54, kisspeptin-14, and kisspeptin-13.

**Pharmacology of Rat and Human GPR54—**After identifica...
tion of these peptides, human GPR54 cDNA (accession number AY029541) was cloned by PCR from human hypothalamus. The human receptor is 80.5% identical to rat GPR54, with most differences located within the N-terminal and C-terminal domains as well as in the third extracellular loop. The predicted transmembrane segments share 98% identity (Fig. 2). Human GPR54 was expressed in a Gt/H9251 and apo-aequorin-expressing CHO-K1 cell line. We synthesized kisspeptin-54, kisspeptin-14, and kisspeptin-13 as well as a shorter variant designated kisspeptin-10 (YNWNSFGLRF-NH2, the shortest truncated peptide containing a tyrosine available for iodination), and their biological activity on GPR54 was confirmed. Non-ami-
dated peptides were inactive (data not shown). Kisspeptin-10 was iodinated, and binding experiments were performed on CHO-K1 cells expressing rat or human GPR54. Saturation binding using 125I-kisspeptin-10 (Fig. 5 and not shown for human GPR54) revealed a single high affinity binding site (Kd of 1.0 \pm 0.1 and 1.9 \pm 0.4 nM and Bmax of 34.2 \pm 1.9 and 2.4 \pm 0.3 fmol/10^6 cells for rat and human GPR54, respectively). In competition binding experiments, kisspeptin-54, kisspeptin-14, kisspeptin-13, and kisspeptin-10 were equipotent on both the rat and human receptors (Fig. 5, B and C). For the rat receptor, Ki values were 1.81 \pm 0.05, 2.04 \pm 0.03, 2.08 \pm 0.04, and 1.59 \pm 0.07 nM for, respectively, kisspeptin-54, -14, -13, and -10. For the human receptor, Ki values were 1.45 \pm 0.1, 1.65 \pm 0.15, 4.23 \pm 0.10, and 2.33 \pm 0.13 nM for, respectively, kisspeptin-54, -14, -13, and -10. In the aequorin-based functional assay, the three pep-
tides were also equally potent on both rat and human GPR54 (Fig. 5, D and E). For the rat receptor, EC50 values were 1.39 \pm 0.03, 1.33 \pm 0.01, 1.38 \pm 0.02, and 1.17 \pm 0.02 nM for, respectively, kisspeptin-54, -14, -13, and -10. For the human receptor, EC50 values were 5.47 \pm 0.03, 7.22 \pm 0.07, 4.62 \pm 0.02, and 4.13 \pm 0.02 nM for, respectively, kisspeptin-54, -14, -13, and -10. The higher EC50 of the peptides for human GPR54 are attributed to the lower expression in the recombinant cell line. Other pep-
tides with RF-amide C termini, including neuropeptide FF, neu-
ropeptide AF, and prolactin-releasing peptide did not elicit func-
tional responses up to 1 \mu M concentrations (data not shown).

Intracellular Coupling of GPR54—To determine the natural coupling properties of the receptor to intracellular signaling pathways, CHO-K1 cell lines expressing human or rat GPR54 but not Gt/H16 or apo-aequorin were established. Kisspeptin-10
stimulated Ca\(^{2+}\) mobilization and phosphatidylinositol turnover in these cells (Fig. 6, A and B). This effect was not affected by pertussis toxin pretreatment. No modification of cAMP accumulation was observed after stimulation of the rat or human GPR54 (data not shown). These results suggest that GPR54 is coupled with proteins of the G\(_{q}\) class. Kisspeptins also stimulated arachidonic acid release from CHO cells expressing rat or human GPR54 in a concentration-dependent manner (Fig. 6, C and D).

Given the previous description of KiSS-1 as a tumor suppressor gene, we evaluated the effects of kisspeptins on cell proliferation and cytoskeleton organization. In CHO cells expressing human or rat GPR54, we observed a strong and sustained stimulation of phosphorylation of the MAP kinases ERK1 and ERK2 (Fig. 6, E and F). We also observed a weak stimulation of p38 MAP kinase phosphorylation (Fig. 6G) but not of stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) (Fig. 6H). However, despite the involvement of the MAP kinase pathways in the control of cell cycle and proliferation, culturing recombinant CHO cells in the presence of kisspeptin-10 resulted in a strong reduction of cell proliferation (Fig. 7). The peptide had no effect on untransfected CHO-K1 cells. No evidence of cell death by apoptosis was found either by flow cytometry using phycoerythrin-conjugated annexin V or a genomic DNA fragmentation assay (data not shown).

Actin microfilament reorganization in recombinant CHO-K1 cells was monitored using phycoerythrin-labeled phalloidin and confocal microscopy. Kisspeptin-10 stimulated stress fiber formation in CHO cells expressing rat GPR54 (Fig. 6I). This effect was abolished in cells pretreated with C3 exoenzyme, suggesting that kisspeptin-10 stimulated stress fiber formation through activation of the Rho subfamily of G proteins. Identical results were obtained with cells expressing human GPR54, whereas kisspeptins had no effects on untransfected CHO-K1 cells (not shown).

**In Vivo Activity of Kisspeptins—** We have started to investigate the function of GPR54 and its ligands in vivo. Given the high expression of GPR54 in hypothalamus and pituitary, we postulated that it could be involved in the control of hormone release. We tested in a first approach the effect of kisspeptin-10 onto oxytocin plasma level in mature female rats. Administration of 25 \(\mu\)g of kisspeptin-10 through the tail vein promoted a significant increase of oxytocin with a maximum 30 min after the injection (Fig. 8).

**DISCUSSION**

GPR54 is an orphan G protein-coupled receptor structurally related to neuropeptide receptors and, particularly, to galanin receptors. The rat and human cDNAs encoding GPR54 were cloned on the basis of sequences available in the data bases. As for most G protein-coupled receptors, predicted transmembrane segments displayed high interspecies conservation. Low similarity was, however, found in the third extracellular loop and the C-terminal intracellular domain (Fig. 2), suggesting that these regions do not play essential roles in the receptor’s function. We determined the distribution of the receptor in

---

**Fig. 5. Pharmacology of rat and human GPR54.** A, saturation binding assay using a CHO-K1 cell line expressing rat GPR54 and \(^{125}\)I-kisspeptin-10 as tracer. Nonspecific binding was determined by using 500 nM unlabeled kisspeptin-10 as competitor. B, competition binding assay on rat GPR54 using different peptides as competitors. C, competition binding assay on human GPR54. D, aequorin-based functional assay using CHO-K1 cells coexpressing rat GPR54, apo-aequorin and Go_{\alpha}. E, aequorin-based assay for human GPR54. Graphs (mean and S.E. of triplicate data points) are representative of at least three independent experiments.
human tissues by RT-PCR. GPR54 was found to be expressed in most tissues tested, including in the central nervous system, gut, and endocrine organs. The highest expression was found in pituitary and placenta. This distribution pattern was used to select the tissues in which the potential ligands of GPR54 could be present. No convincing biological activities were detected in extracts from brain, pituitary, and gut segments, whereas a strong biological activity was detected in a human placenta extract, allowing purification of the active compounds from this source. This led to the identification of kisspeptins, peptides encoded by the previously described gene KiSS-1.

KiSS-1 was isolated as a tumor metastasis suppressor gene for the human malignant melanoma cell line C8161 by a sub-traction cloning method (16–18). The predicted product of KiSS-1 is a 145-amino acid protein with a putative signal sequence. The secretory nature of the protein was, however, not demonstrated so far. KiSS-1 does not share similarities with any other protein in the data bases. The metastasis potential of melanoma and breast cancer cell lines in vivo was suppressed after KiSS-1 transfection without affecting the proliferation and migration properties of the cells (16, 19). KiSS-1 was also reported to inhibit MMP-9 production when transfected in HT1080 cells (20). The molecular mechanisms underlying these observations were, however, not identified.

Three peptides of 54, 14, and 13 amino acids were identified within the active fractions, and their biological activity was confirmed for the synthetic peptides. The long peptide (54 amino acids) is predicted to result from the proteolytic process-ing of KiSS-1 by furin or prohormone convertases, as its se-quence is surrounded by pairs of basic residues in the full-size protein (Fig. 4). No obvious cleavage sites were found that would result in the generation of the two shorter peptides. Their presence in the same fraction as the longer peptide sug-gested that the 54-amino acid peptide might be unstable and degraded into the shorter peptides during the extraction pro-cess, or even during mass spectrometry analysis. In binding and functional assays, it was found that kisspeptin-54, -14, and -13 as well as a shorter peptide designated kisspeptin-10 had the same affinity and efficacy on the rat receptor. This indi-cates that the C-terminal part of the peptides is responsible both for the high affinity binding and the activation of GPR54. The major role of the RF-amide moiety was demonstrated by
stress fiber formation in GPR54-expressing cells through Rho activation. This observation might be linked to an earlier report describing faster spreading to culture plates of breast cancer cells transfected with KiSS-1 cDNA (19), although expression of GPR54 was not investigated in these cells. After completion of this study, the identification of kisspeptins as ligands of GPR54 was reported by Ohtaki et al. (23) and Muir et al. (24).

In conclusion, we have isolated KiSS-1-derived peptides from human placenta as high affinity natural agonists of a previously orphan G protein-coupled receptor designated GPR54. Tissue distribution suggested that GPR54 might be implicated in various hormonal function, a hypothesis supported by the demonstration that KiSS-1-derived peptides stimulate oxytocin release in rats. It is, however, likely that many other biological actions of kisspeptins in vivo will be uncovered in the future. The correlation of the present observations with the previous description of KiSS-1 as a tumor metastasis suppressor gene will require further investigations.

Acknowledgment—We thank Dominique Revets for technical assistance.

REFERENCES

1. Bockaert, J., and Pin, J. P. (1999) EMBO J. 18, 1723–1729
2. Civelli, O., Nothacker, H. P., Saito, Y., Wang, Z., Lin, S. H., Reinscheid, R. K. (2001) Trends Neurosci. 24, 230–237
3. Meunier, J. C., Molle, E., Toll, L., Suaudeau, C., Moisand, C., Alvinerie, P., Bocquet, J. L., Guillemot, J. C., Ferrara, P., and Monsarrat, B. (1995) Nature 377, 532–535
4. Reinscheid, R. K., Nothacker, H. P., Bourson, A., Henningsen, R. A., Bunzow, J. R., Grandy, D. K., Langen, H., Monsma, F. J., Jr., and Civelli, O. (1995) Science 270, 792–794
5. Sakurai, T., Amemiyabi, A., Ishii, M., Matsuoka, I., Chemelli, R. M., Tanaka, H., Williams, S. C., Richardson, J. A., Kozlowski, O. F., Wilson, S., Arch, J. R., Buckingham, R. S. K., Haynes, A. C., Carr, S. A., Annan, R. S., McNaught, D. E., Liu, W. S., Terret, J. A., Elshourbagy, N. A., Bergsma, D. J., and Yanagisawa, M. (1998) Cell 92, 573–585
6. Chemelli, R. M., Willie, J. T., Sinton, C. M., Elmquist, J. K., Scammell, T., Lee, C., Richardson, J. A., Williams, S. C., Xiong, Y., Kisanuki, Y., Fitch, T. E., Nakazato, M., Hammer, R. E., Saper, C. B., and Yanagisawa, M. (1999) Cell 98, 437–451
7. Hinuma, S., Habata, Y., Fujii, R., Kawamata, Y., Hosoya, M., Fukusumi, S., Kitada, C., Masuo, Y., Asano, T., Matsumoto, H., Sekiguchi, M., Kurokawa, T., Nishimura, O., Hnda, H., and Fujino, M. (1998) Nature 393, 272–276
8. Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Masteu, H., and Kangawa, K. (1999) Nature 402, 656–660
9. Tatamoto, K., Hosoya, M., Habata, Y., Fujii, R., Kayekawa, T., Tosp, M. X., Kawanuma, Y., Fukuwama, S., Hinuma, S., Kitada, C., Kurokawa, T., Hnda, H., and Fujino, M. (1998) Biochem. Biophys. Res. Commun. 251, 471–476
10. Lee, D. K., Nguyen, T., O’Neill, G. P., Cheng, R., Liu, Y., Howard A. D., Coulou, N., Tan, C. P., Tang-Nguyen, A. T., George, S. R., and O’Dowd, B. F. (1999) FEBS Lett. 446, 103–107
11. Kotani, M., Molle, E., Dethue, M., Le Pou, E., Brézillon, S., Vakili, J., Mazargul, H., Vassart, G., Zajac, J. M., and Parmentier, M. (2001) Br. J. Pharmacol. 133, 138–144
12. Blanpain, C., Doranz, B. J., Vakili, J., Rucker, J., Govaerts, C., Baik, S. S. W., Lorthioir, O., Migette, I., Libert, F., Baleux, F., Vassart, G., Dom, R. W., and Parmentier, M. (1999) J. Biol. Chem. 274, 34719–34727
13. Langmead, C. J., Swekere, P. G., Chambers, J. R., Batcliffe, S. J., Jones, D. N., Hirst, W. D., Price, G. W., and Herdon, H. J. (2000) Br. J. Pharmacol. 131, 683–688
14. Gijon, M. A., Spencer D. M., Siddiqui, A. R., Bonventre, J. V., and Leslie, C. C. (2000) J. Biol. Chem. 275, 20146–20156
15. Deleted in proof
16. Lee, J. H., Miele, M. E., Hicks, D. J., Phillips, K. T., Tract, J. M., Weissman, R. E., and Welch, D. R. (1996) J. Natl. Cancer Inst. 88, 1701–1707
17. Lee, J. H., and Welch, D. R. (1997) Int. J. Cancer 71, 1035–1044
18. West, A., Vujta, J. P., Welch, D. R., and Weissman, B. E. (1996) Genomics 54, 145–148
19. Lee, J. H., and Welch, D. R. (1997) Cancer Res. 57, 2384–2387
20. Yan, C., Wang, H., and Boyd, D. D. (2001) J. Biol. Chem. 276, 1164–1172
21. Guillelmet, L., Levy, A., Zhao, Z. J., Berezitat, G., and Rothu, B. (2000) J. Biol. Chem. 275, 28349–28358
22. Thibonner, M., Conarty, D. M., and Plesnicher, C. L. (2000) Am. J. Physiol. Heart Circ. Physiol. 279, 2529–2539
23. Ohtaki, T., Shintani, Y., Honda, S., Matsumoto, H., Hori, A., Kanehaka, H., Terao, Y., Kuman, S., Takatsu, Y., Masuda, Y., Ishibashi, Y., Watanabe, T., Asada, M., Yamada, T., Suenaga, M., Kitada, C., Usuki, S., Kurokawa, T., Hnda, H., Nishimura, O., and Fujino, M. (2001) Nature 411, 613–671
24. Muir, A. J., Chamberlin, L. A., Michalovich, D., Moore, D. J., Calamari, A., Zeker, G. P. S., Sarai, H. M., Chambers, J. K., Murdock, P., Steplewski, K., Shabon, U., Miller, J. E., Middleton, S. E., Darker, J. G., Larmine, C. G. C., & Wilson, S., Bergsma, D. J., Emson, P., Faull, R., Philpott, K. L., and Harrison, D. C. (2001) J. Biol. Chem. 276, 28969–28975
The Metastasis Suppressor Gene KiSS-1 Encodes Kisspeptins, the Natural Ligands of the Orphan G Protein-coupled Receptor GPR54
Masato Kotani, Michel Detheux, Ann Vandenbogaerde, David Communi, Jean-Marie Vanderwinden, Emmanuel Le Poul, Stéphane Brézillon, Richard Tyldesley, Nathalie Suarez-Huerta, Fabrice Vandeput, Cédric Blanpain, Serge N. Schiffmann, Gilbert Vassart and Marc Parmentier

J. Biol. Chem. 2001, 276:34631-34636.
doi: 10.1074/jbc.M104847200 originally published online July 16, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M104847200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 23 references, 7 of which can be accessed free at
http://www.jbc.org/content/276/37/34631.full.html#ref-list-1