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# Molecular cloning and characterization of a novel Gq-coupled orphan receptor GPRg1 exclusively expressed in the central nervous system

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**Abstract**

G-protein-coupled receptors (GPCRs) are important mediators of signal transduction and are therefore potential targets for pharmacological therapeutics. Here, we report the identification and characterization of an orphan GPCR, termed GPRg1, which was found in the GenBank database following searches with GPCR query sequences. Quantitative PCR analysis revealed that GPRg1 transcripts are expressed almost exclusively in the brain. Moreover, in situ hybridization experiments in brain demonstrated that GPRg1 is abundantly expressed in the ventrolateral region of caudate putamen, the habenular nucleus, the zona incerta, and the medial mammillary nucleus. In addition, overexpression of GPRg1 in 293-EBNA cells activates serum response factor mediated transcription, which was completely inhibited by the Gq/11 selective inhibitor YM-254890, indicating the coupling of GPRg1 with Gq/11. These findings suggest that GPRg1 is a candidate receptor for novel physiologically bioactive substrates and that it plays important roles in the central nervous system.


*Keywords:* Orphan GPCR; Rhodopsin family; Caudate-putamen; Nucleus; Zona incerta; Gq/11-coupled receptor

The GPCR family of genes are widely expressed in different human tissues and play fundamental roles in human physiological as well as pathophysiological conditions. Both the GPCR and the heterotrimeric G proteins, consisting of Gα, β, and γ subunits, transduce extracellular stimuli such as ion exchanges, organic odorants, amines, peptides, proteins, lipids, nucleotides, and photons [1–3]. The activation of GPCR by agonists induces the exchange of GDP for GTP on the coupled Gα-subunit, resulting in Gα-GTP complex dissociation from the Gβγ-subunit and activation of downstream effectors. This in turn regulates various functions such as gene transcription, mitogenesis, metabolism, muscle contractile states, and ion channel regulation. Four classes of G protein α subunits, Gq/11, Gs, Gi, and G12/13, are involved in signal transduction. The Gs and Gi subunits regulate adenyl cyclase activity, whereas the Gq/11 subunit regulates phospholipase C activity and the G12/13 subunit regulates small G protein RhoA activity.

Based on the sequence motifs in their primary structures, mammalian GPCRs can be classified into three major subfamilies, Class A (rhodopsin-like), Class B

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(secretin-like), and Class C (metabotropic glutamate-like), and into some minor subfamilies [4,5]. In addition, genome sequence analysis indicates that the number of human GPCRs that exist for endogenous ligands, and for the sensory system such as odors and tastes, is approximately predicted to be 370 and 400 receptors, respectively [6]. Among the 370 endogenous ligand GPCRs, the substrates of about 100 have not yet been identified and these GPCRs are referred to as "orphan" receptors [7]. Significantly, it has been estimated that more than half of all modern drugs are GPCR targets, particularly among the 370 endogenous ligand receptors [8], and thus the elucidation of the remaining orphan GPCRs has considerable potential for developing new treatments for human diseases.

In the present study, we describe the cloning and expression patterns of a novel Gq-coupled orphan GPCR, termed GPRg1, which belongs to the rhodopsin family of GPCRs. GPRg1 is expressed preferentially in distinct regions of the central nervous system (CNS), suggesting that it has specific roles in the modulation of brain functions.

## Materials and methods

*Reagents.* The Rho-kinase inhibitor, Y-27632, was purchased from BioMol. YM-254890 was isolated from the culture broth of *Chromobacterium* sp. QS3666 [9,10]. $Arg^8$-Vasopressin (AVP) was purchased from PEPTIDE. The pSRE (c-fos serum response element)-luc and pCRE (cAMP response element)-luc were obtained from Stratagene. The phRL-TK and dual-luciferase reporter assay system were purchased from Promega. LipofectAMINE 2000, Dulbecco's modified Eagle's medium (DMEM) and 293-EBNA cells were purchased from Invitrogen. PGEM-T easy vector was purchased from Promega. Receptor-Gq interaction inhibitory "minigene" (Gq-I), which corresponds to the C-terminal peptide sequence of Gq residues 305–359, was cloned into pcDNA3 [11]. The pEF-BOS vector was a gift from Prof. S. Nagata (Osaka university Medical School, Osaka, Japan).

*Cloning of human, rat, and mouse GPRg1 and human GPRg1b.* The human GPRg1 (hGPRg1) coding gene was discovered in the HTGS (high-throughput genome sequence) division of GenBank by performing a BLAST search [12] with known GPCRs as query sequences, using a previously described method [13]. The full-length cDNA of hGPRg1 was amplified using a 5′- and 3′-rapid amplification of cDNA ends (RACE) system from fetal brain Marathon-ready cDNA (Clontech) as described previously [14]. Mouse GPRg1 (mGPRg1), rat GPRg1 (rGPRg1), and human GPRg1b (hGPRg1b) were also identified using a BLAST search of GenBank based on their homology to hGPRg1 and full-length cDNAs were again obtained by RACE. All of the isolated cDNAs encoding full-length open reading frame were cloned into the *Xba*I site of pEF-BOS-dhfr [15].

*Tissue distribution studies.* Tissue distribution studies for hGPRg1 and hGPRg1b were undertaken using a previously described method [16]. Briefly, human poly(A)$^+$ RNA from various human tissues (Biochain or Clontech) was subjected to RT-PCR. The tissue expression levels of hGPRg1 and hGPRg1b were quantitatively analyzed using a Prism 7700 Sequence Detector (Applied Biosystems) with the primer sets (5′-TGCGGCTTGGGTTTCG-3′ and 5′-GGTAAACCG AGGCACAGCAA-3′) and (5′-TTCTACTGGTGGCTGGACAT GT-3′ and 5′-ACTTGAGGACCTCGTCCAGTGT-3′), respectively.

*In situ hybridization.* In situ hybridization experiments were performed as described previously [17]. Serial coronal sections (30 μm thick) of mouse brain were prepared using a cryostat and the primers 5′-ACAAGCTTAGGAGAAAGAGC-3′ and 5′-GGGTCACGGG-GATACTTTT-3′ were used to amplify probe DNA for mGPRg1, which was then subcloned into a PGEM-T easy vector. $^{35}$S-labeled sense and antisense probes, corresponding to nucleotide numbers 599–1041 of mGPRg1, were transcribed using T7 or SP6 RNA polymerases. Antisense probes produced specific hybridization signals for mGPRg1 whereas the sections hybridized with the sense probes exhibited no specific signal (data not shown).

*Signal pathway studies using reporter gene assays.* 293-EBNA cells were seeded onto collagen-coated 96-well plates (15,000 cell/well) in DMEM containing 1% fetal bovine serum (FBS) for 18 h, and either 10 ng/well hGPRg1, V1a vasopressin receptor or V2 vasopressin receptor in pEF-BOS-dhfr was co-transfected with 10 ng/well pSRE-luc or pCRE-luc and 5 ng/well phRL-TK, using LipofectAMINE 2000. To determine the effects of YM-254890 and Y-27632 on the GPRg1 signal, after 2 h of DNA transfection, the cells were treated with or without YM-254890 (1 μM) or Y-27632 (3 μM) for 24 h and lysed. To determine the effects of YM-254890 on the V1a and V2 receptor signals, after 18 h of DNA transfection, the cells were treated with AVP (100 nM) with or without YM-254890 (1 μM) or Y-27632 (3 μM) for 6 h and lysed. Cell lysates were analyzed using the dual-luciferase reporter assay system and a model ML3000 luminometer (Dynatech).

## Results

### *Cloning of human, mouse, and rat GPRg1 and human GPRg1b*

We identified the human GPRg1 gene (hGPRg1, Accession No. AB196529) from the genomic database by BLAST analysis with known GPCRs. We then cloned the full-length cDNA of GPRg1 by 5′- and 3′-RACE of human fetal brain cDNA. Further BLAST analysis of the human genomic DNA database indicated that the hGPRg1 gene is localized to chromosome 16p12.3 and that its ORF is composed of two exons (1st exon, nucleotide numbers 1–127; and 2nd exon, nucleotide numbers 128–10629). We then identified the coding region of mouse GPRg1 (mGPRg1, Accession No. AB196531) and rat GPRg1 (rGPRg1, Accession No. AB196532) from the corresponding genomic databases. Both of these rodent genes encode 345 amino acids, have 100% identity in their primary structures, and share 96% amino acid identity to hGPRg1 (Fig. 1A). Furthermore, based on BLAST analysis of the human genome database using hGPRg1 as a query sequence, we identified cDNA from a human testis cDNA library with a predicted amino acid sequence that shares 44% identity to hGPRg1 (Fig. 1B). This gene, termed human GPRg1b (hGPRg1b, Accession No. AB196530), was localized to chromosome 17q25.1 and is composed of three exons (1st exon, nucleotide numbers 1–94; 2nd exon, nucleotide numbers 95–253; and 3rd exon, nucleotide numbers 254–1125).

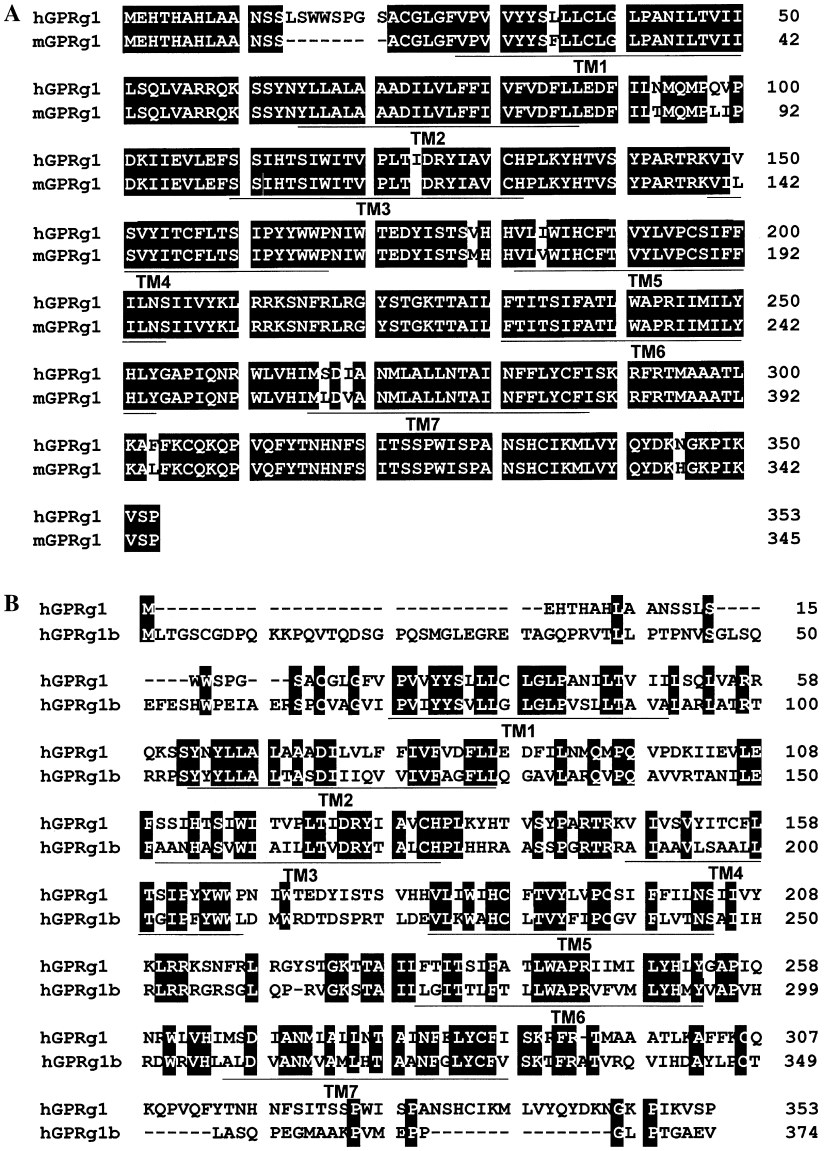


Fig. 1. Amino acid sequence comparisons of human and rodent GPRg1. (A) Amino acid alignment of hGPRg1 and mGPRg1. (B) Amino acid alignment of hGPRg1 and hGPRg1b. Identical amino acids are shadowed and putative transmembrane regions (TM1–7) are underlined. The nucleotide sequence data reported here have been submitted to DDBJ, EMBL, and GenBank nucleotide sequence databases with Accession Nos. AB196529 (hGPRg1), AB196530 (hGPRg1b), AB196531 (mGPRg1), and AB196532 (rGPRg1).

*Tissue distribution study*

The human tissue distribution of GPRg1 and GPRg1b was studied using quantitative PCR (Q-PCR) based analysis. Human GPRg1 was found to be abundantly expressed in the fetus and adult CNS but was detected at very low levels in the peripheral tissues (Fig. 2A). Within the CNS, GPRg1 was mainly expressed in the caudate putamen (CP) and hypothalamus. In contrast, hGPRg1b gene expression was observed to be widely distributed in peripheral tissues as well as in the CNS (Fig. 2B). The strongest expression of hGPRg1b was detected in the thyroid gland, with moderate expression observed in the corpus callosum and cerebellum within the CNS, and in the spleen, small intestine, and pancreas among the peripheral tissues (Fig. 2B).

*In situ hybridization*

In situ hybridization using a GPRg1 cDNA probe revealed specific CNS labeling (Fig. 2C) and expression of GPRg1 in some distinct regions. The olfactory bulb, cerebral cortex, and hippocampus did not show significant signals but in the CP, GPRg1 was found to be strongly expressed in the lateral region, but very weakly in the medial region. In the lateral septum, moderate levels of expression were evident and in the diencephalon, the medial habenular nucleus showed the strongest GPRg1 signal

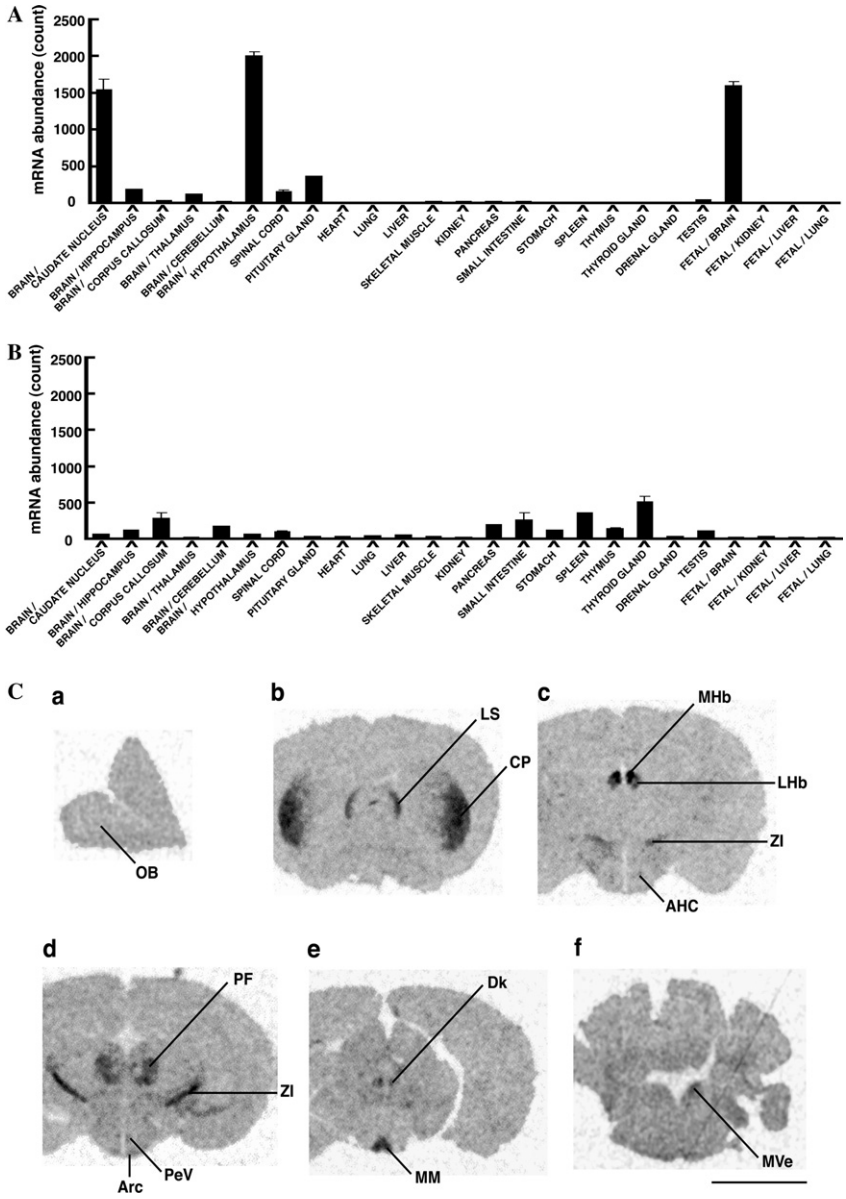


Fig. 2. Distribution of GPRg1 and GPRg1b transcripts in human tissues. Analyses were undertaken with gene-specific primer sets for hGPRg1 (A) and hGPRg1b (B). Each bar represents the mean value of duplicate data determinations from a single repeated experiment. (C) Series of low power photomicrographs of autoradiograms, arranged from rostra to caudal, showing the overall distribution of GPRg1 mRNA expression in the mouse brain. OB, olfactory bulb; LS, lateral septum; CP, caudate-putamen; MHb, medial habenular nucleus, LHb, lateral habenular nucleus; ZI, zona incerta; AHC, anterior hypothalamic area, central part; PF, parafascicular thalamic nucleus; Arc, arcuate hypothalamic nucleus; PeV, periventricular hypothalamic nucleus; Dk, nucleus Darkschewitsch; MM, medial mammillary nucleus, median part; and MVe, medial vestibular nucleus. Bar = 3 mm.

within the brain, whereas the lateral habenular nucleus showed only a moderate signal. In the thalamus, the parafascicular thalamic nucleus also had a moderate GPRg1 signal, whilst the other thalamic region did not show any specific labeling. The zona incerta showed a moderate signal. In the hypothalamus, the medial mammillary nucleus was strongly labeled but the anterior hypothalamic area, paraventricular hypothalamic nucleus, and periventricular hypothalamic nucleus had only a weak GPRg1 signal. In the brainstem, the nucleus of Darkschewitsch in the midbrain and medial vestibular nucleus in the pons were also weakly labeled.

### *Signal transduction pathway of GPRg1*

To elucidate the signal transduction pathways mediated by GPRg1, we utilized SRE and CRE reporter assay experiments. We overexpressed GPRg1 in 293-EBNA cells and examined the effects of inhibiting specific classes of G-protein-mediated SRE and CRE

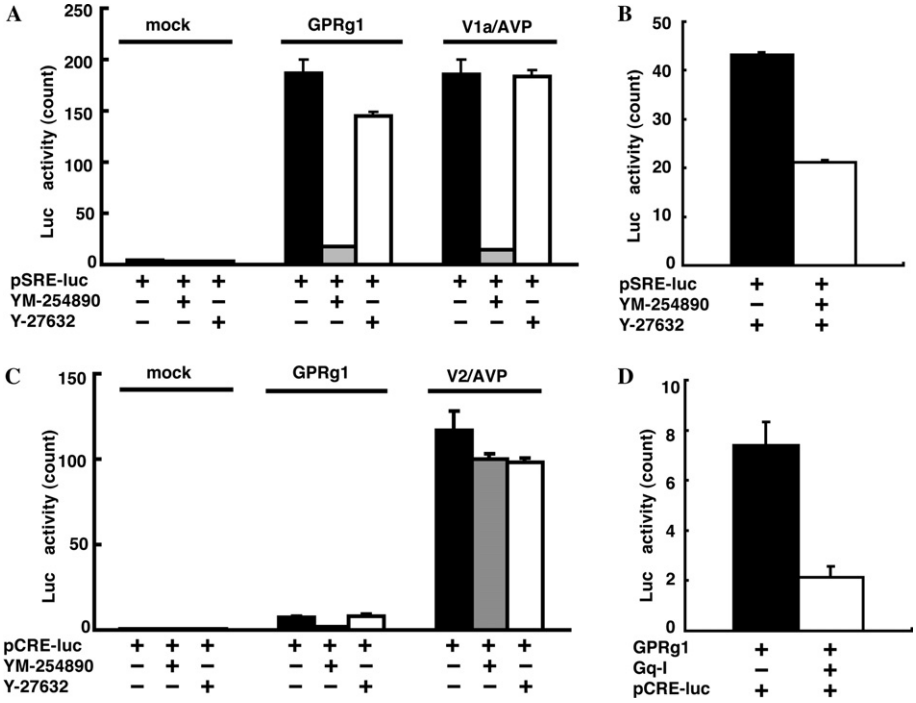


Fig. 3. The effects of YM-254890, Y-27632, and Gq-I upon GPRg1-induced constitutive activity. (A,C) Effects of YM-254890 and Y-27632 on GPRg1-mediated pSRE-luc activity (A) and pCRE-luc activity (C). GPRg1, cloned into pEF-BOS-dhfr, or a control vector was co-transfected with either pSRE-luc or pCRE-luc into 293-EBNA cells. Following transfection, the cells were treated without (black bar) or with YM-254890 (1 μM, gray bar) or Y-27632 (3 μM, white bar). In addition, V1a (A) or V2 (B), cloned into pEF-BOS-dhfr, were co-transfected with either pSRE-luc or pCRE-luc. After 18 h, the cells were treated with or without YM-254890 (1 μM) or Y-27632 (3 μM) and then stimulated with AVP (100 nM). The luciferase activities of the control vector with AVP (100 nM)-mediated pSRE-luc and pCRE-luc activity were measured at $4.7 \pm 0.05$ and $0.6 \pm 0.03$, respectively. (B,D) The effects of Gq-I on constitutive GPRg1-mediated pSRE-luc and pCRE-luc activities. GPRg1, expressed from the pEF-BOS-dhfr and pSRE-luc (B) or pCRE-luc (D) vectors, was co-transfected without (black bar) or with (white bar) Gq-I (in pcDNA3.1). The data represent means ± SD of triplicate determinations from a single experiment performed three times.

activation, measured by luciferase expression. GPRg1 expression in 293-EBNA cells produced higher levels of SRE activity than the control vector (Fig. 3A). Generally, two classes of G proteins, Gq/11 and G12/13, induce the activation of SRE via the activation of SRF [18]. To determine which of these G proteins activated SRE in our reporter system, we treated the cells with either YM-254890 (Gq/11-selective inhibitor) [9,10] or Y-27632 (G12/13 signal blocker by Rho-kinase inhibition) [19]. It was subsequently found that GPRg1-induced SRE-mediated gene transcription was almost completely suppressed by treatment with YM-254890, but not with Y-27632 (Figs. 3A and C). This indicated that the activation of SRE-luciferase by GPRg1 overexpression is mediated by Gq/11. In addition, co-transfection of Gq-I (a minigene that selectively inhibits receptor-Gq interaction [11]) significantly blocked constitutive activation of SRE, further indicating the involvement of Gq/11 in the activation of SRE (Fig. 3B). Similar results were obtained following transfection of a Gq/11-coupled receptor, the V1a vasopressin receptor (V1a). Increased SRE activity, induced by overexpression of V1a stimulated with AVP, was blocked by YM-254890 but not by Y-27632 (Fig. 3A).

We next examined whether GPRg1 also activates CRE in 293-EBNA cells (Fig. 3C) and found that overexpression of GPRg1 indeed induces CRE at significant levels. The GPRg1-induced CRE activity was completely inhibited by treatment with YM-254890, but not by treatment with Y-27632 (Fig. 3C), suggesting that CRE activation is mediated by Gq. This was further supported by our finding that transfection of Gq-I significantly reduced CRE-mediated constitutive activities (Fig. 3D). In contrast however, the inhibition of CRE activity by YM-254890 was not observed for the Gs-coupled V2 vasopressin receptor. When stimulated by AVP, strong CRE activation was produced by the overexpression of V2 but this was not blocked significantly by either YM-254890 or Y-27632. Hence, these results suggest that GPRg1 is coupled with the Gq/11 protein which leads to both SRE and CRE activation.

## Discussion

In the present study, we have identified a novel GPCR, termed GPRg1, containing a D-R-Y motif at

the end of its TM3 that is characteristic of the rhodopsin family of GPCRs [4,20]. Additionally, the third exon of hGPRg1b is identical in sequence to GPR142, which was recently identified using computer-based human genomic DNA analysis [21], but the primary structure of the N-terminal region and the overall amino acid sequence (hGPRg1b: 374 and GPR142: 462) were different between hGPRg1b and GPR142. Presently, we have no clear explanation for this and the fact that we could not obtain the corresponding GPR142 cDNA during our cloning experiments from cDNA libraries suggests that GPR142 might be incorrectly predicted from the computer-based exon–intron analysis.

BLAST analysis of the SWISS-PROT protein sequence database revealed that hGPRg1 shares high homology with the thyrotropin-releasing hormone receptor (SWISS-PROT Accession No. P34981), the nociceptin receptor (Accession No. P41146), the C-X-C chemokine receptor type 4 (Accession No. P61073), and the B1 bradykinin receptor (Accession No. P46663). Whereas these peptide receptors show less than 20% homology in amino acid sequence with GPRg1, GPRg1b has a much stronger sequence similarity to GPRg1 with 44% homology between the respective amino acid sequences. The strong homology between GPRg1 and GPRg1b, and weak homology with other GPCRs suggests that GPRg1 and GPRg1b form a distinct subbranch of the rhodopsin-type GPCR subfamily. In spite of this, however, these two receptors have quite different tissue expression profiles, indicating that they have distinct functions, but we postulate that they are also candidates for novel physiologically bioactive peptide–substrate pathways and play important roles in distinct organs.

We observed strong activation of SRE-mediated gene transcription after overexpression of GPRg1 in a gene reporter assay experiment. It has been previously shown that certain classes of G-protein induce SRE activation through distinct signaling pathways. Gq/11-coupled receptors activate both phospholipase C (PLC) and PKC, and subsequently induce SRE-mediated gene transcription [18]. G12/13 also drives SRE-mediated gene transcription via the RhoA signaling pathway [22]. In the present study, the Gq-selective inhibitor, YM-254890, and the receptor/Gq interaction inhibitor, Gq-I, significantly suppressed SRE-mediated gene transcription. In contrast, the Rho-selective inhibitor, Y-27632, did not inhibit SRE-mediated gene transcription by GPRg1. Therefore, it is highly probable that GPRg1 is coupled with Gq and that this regulates the activation of SRE.

Transcriptional activation of CRE also occurred following the overexpression of GPRg1. The expression of CRE is mediated by the CRE-binding protein (CREB), which is phosphorylated by various serine/threonine kinases [23,24]. In our study, it is likely that the activation of CRE occurred via Gq rather than Gs. Our current reporter studies showed that GPRg1-mediated CRE luciferase activation was suppressed by both YM-254890 and Gq-I. Moreover, GPRg1 cDNA transfected in 293-EBNA cells did not show a dose-dependent increase in intracellular cAMP accumulation (data not shown). This finding contrasted with the results of Gs-coupled V2 receptor stimulated with AVP, in which CRE activation was not suppressed by YM-254890. The mechanism by CRE activity is upregulated after the overexpression of GPRg1 is still not yet clear however. The absence of cAMP accumulation suggests that there may be a PKA-independent CREB phosphorylation pathway. Activation of Gq leads to the production of inositol-1,4,5-trisphosphate, which stimulates the release of $Ca^{2+}$ from intracellular stores [25,26]. Phosphorylation of CREB by calcium/calmodulin-dependent protein kinase, activated by increased cytosolic calcium mobilized by GPRg1, might therefore be the mechanism underlying CRE activation [23].

One interesting property of GPRg1 is that it is expressed almost exclusively in the CNS. Even in brain however, GPRg1 mRNA expression is confined to several functionally distinct regions. In situ hybridization analyses demonstrated the abundant expression of GPRg1 mRNA in CP, although this is confined to the ventrolateral region. The CP is regionally divided into several areas based on the localization of cortical projection [27] and the pattern of GPRg1 mRNA expression significantly corresponds to the projection from the somatosensory cortex. The significance of dopaminergic projection from the substantia nigra to the caudate-putamen has been intensively investigated [28]. The dopamine D2 receptor, abundantly expressed in the neurons of the CP, is regarded as an important regulator of locomotor activity [29]. In recent studies, the activity of the D2 receptor has been suggested to be negatively regulated by heterologous desensitization through the activation of Gq-coupled GPCRs, which can phosphorylate the D2 receptor through a PKC-mediated mechanism [30]. The cholecystokinin $CCK_2$ and neurotensin NT1 receptors, which are coupled with Gq, have also been reported to be candidates as negative modulators of the D2 receptor [31,32]. It is possible therefore that GPRg1 is also involved in the modulation of locomotor activity.

In conclusion, we have identified a novel orphan GPCR, termed GPRg1, and report its distribution and some of its properties. This receptor is expressed preferentially in the distinct regions of the CNS in mouse brain, suggesting its importance in the regulation of specific brain functions. We also demonstrate the coupling of Gq/11 with GPRg1, which might be a useful finding for the future development of cellular systems for the screening of endogenous ligands and/or agonist/antagonist of GPRg1.

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