Identification and Characterization of a Novel RF-amide Peptide Ligand for Orphan G-protein-coupled Receptor SP9155*

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Orphan G-protein-coupled receptors are a large class of receptors whose cognate ligands are unknown. SP9155 (also referred to as AQ27 and GPR103) is an orphan G-protein-coupled receptor originally cloned from a human brain cDNA library. SP9155 was found to be predominantly expressed in brain, heart, kidney, retina, and testis. Phylogenetic analysis shows that SP9155 shares high homology with Orexin, NPF, and cholecystokinin (CCK) receptors, but identification of the endogenous ligand for SP9155 has not been reported. In this study, we have used a novel method to predict peptides from genome data bases. From these predicted peptides, a novel RF-amide peptide, P52 was shown to selectively activate SP9155-transfected cells. We subsequently cloned the precursor gene of the P52 ligand and characterized the activity of other possible peptides encoded by the precursor. This revealed an extended peptide, P518, which exhibited high affinity for SP9155 \( (EC_{50} = 7\text{ nM}) \). mRNA expression analysis revealed that the peptide P518 precursor gene is predominantly expressed in various brain regions, coronary arteries, thyroid and parathyroid glands, large intestine, colon, bladder, testes, and prostate. These results indicate the existence of a novel RF-amide neuroendocrine peptide system, and suggest that SP9155 is likely the relevant G-protein-coupled receptor for this peptide.

G-protein coupled receptors (GPCRs)\(^1\) are members of a large protein family that share a common structural motif of seven transmembrane domains (1, 2). GPCRs mediate a variety of physiological functions by interacting with extracellular ligands including small chemical molecules, peptides, and proteins, then transmitting the signal to intracellular second messengers via G proteins (1, 2). In recent years, the primary sequence of many GPCRs have been identified through searching the human genome sequence database. Whereas these GPCRs have common structural motifs including seven transmembrane domains, often their cognate ligands and biological function are unknown (3–5). To understand the biological functions of these so called orphan receptors, and to enable identification of pharmacological agents active at these receptors, it is helpful to first identify their endogenous cognate ligands (3–5). Currently there are two main approaches generally used to identify orphan receptor ligands. One common approach has been to screen the orphan GPCRs against a collection of known or putative GPCR ligands. This approach has been used successfully to identify several ligand receptor pairings, including MCH, NMU, and P2Y13 etc. (6–8). However, because ligand collections are somewhat limited, they often do not contain the appropriate ligand for the orphan receptor of interest. Another approach involves purification of the ligands from animal tissue extracts. This approach has also been successful in a number of cases including OFQ/Nociceptin, MCH, Orexin, and P2Y12 etc. (9–13). However, purification of ligands from animal tissue extracts is labor-intensive and is complicated by the potential for background activity, the low abundance of ligands, and stability of ligands in animal tissue extracts. In the present study we utilized an alternative approach to identifying ligands for orphan receptors. Because many orphan receptors are predicted to be peptide receptors, and peptide ligands must be encoded by DNA sequences, it should be possible using a variety of methods to predict peptide ligands from sequences stored in the human genome data bases. Peptides predicted in this manner can then be synthesized and tested for biological activity of GPCRs. In the present study, we have used a novel peptide homology algorithm (PepPat) to search the human genome for potential bio-active peptides (16). Using this approach, we have successfully identified a novel ligand for an orphan receptor designated as SP9155.

EXPERIMENTAL PROCEDURES

Reagents and Materials—All chemicals were obtained from Sigma except as otherwise noted. Fluor-3-AM and pluronic acid were from Molecular Probes. Cell culture media and reagents were from Invitrogen. All synthetic peptides were custom synthesized at Invitrogen. All cloning work was performed according to standard procedures in Ref. 24. Chimeric Go proteins \( (G_{\alpha_{q}/G_{\alpha_{o}}}, G_{\alpha_{q}/G_{\alpha_{s}}}, G_{\alpha_{q}/G_{\alpha_{12}}}, G_{\alpha_{q}/G_{\alpha_{15}}}, G_{\alpha_{q}/G_{\alpha_{16}}} ) \) were constructed by replacing the five C-terminal residues of human Go with the five amino acid residues of the corresponding G protein (14), and were cloned into the mammalian expression vector pCDNA3.1 (Invitrogen).

Bioinformatic Identification of Novel RF-amide Peptides—RF-amide peptides share a common C-terminal RFG[KR] motif. First, a virtual transcripts protein data base (VTS) was generated from human genomic sequence using GenScan (20). Using a pattern-based oligopeptide homology search algorithm (PepPat) (16), we searched the VTS protein data base for proteins or exons containing this RFG[KR] motif. Proteins or exons that contain RFG[KR] motifs were further analyzed for the presence of a leader peptide or transmembrane domains (16). Those genes containing at least one RFG[KR] motif, with a predicted leader sequence but without predicted TM domain, were regarded as putative RF-amide precursors. These predicted peptides were then synthesized and put into our in-house peptide collection for screening.

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Cloning and Expression of Human and Mouse SP9155—A full-length cDNA of SP9155 (referred to as AQ27) was originally isolated from a human brain cDNA library and was disclosed in patent WO20001016316. Based on the AQ27 sequence two primers were designed and the full-length PCR product was obtained from human brain cDNA library. This clone was designated as SP9155. The open reading frame of SP9155 was then subcloned into the expression vector pCDNA3.1 (Invitrogen) and used for cell transfection experiments. To identify the mouse homologue of SP9155, the protein sequence of human SP9155 was used to search GenBank™ with BLAST, and two mouse EST sequences (BI729969 and BB084541) were identified that showed homology to SP9155 and were designated as mouse SP9155. This clone was used to search GenBank™ with BLAST, and two human EST sequences (BQ839589 and BI729970) were identified that showed homology to human SP9155. A PCR product was then cloned into TOPO-pCDNA3.1 vector (Invitrogen) and sequence confirmed.

Cloning of Human and Mouse Peptide: P518 Precursor—Primer sets for the human and mouse were designed based on the human genomic sequence for the peptide coding VTS; the 5' and 3' ends of the human open reading frame were based on these 5' and 3' regions (underlined). Clone was designated as SP9155. The open reading frame of SP9155 was then subcloned into the expression vector pCDNA3.1 (Invitrogen) and used for cell transfection experiments. To identify the mouse homologue of SP9155, the protein sequence of human SP9155 was used to search GenBank™ with BLAST, and two mouse EST sequences (BI729969 and BB084541) were identified that showed homology to SP9155 and were designated as mouse SP9155. This clone was used to search GenBank™ with BLAST, and two human EST sequences (BQ839589 and BI729970) were identified that showed homology to human SP9155. A PCR product was then cloned into TOPO-pCDNA3.1 vector and sequence confirmed.

| Primer and probe sequences used in the quantitative PCR reactions | Human | Mouse |
|---------------------------------------------------------------|-------|-------|
| SP9155 | | |
| Forward primer  | 777GAAATGGCATTAAATAGCCAGGAAG  | 851GCGAACACTGAAAGACCTGCG |
| Reverse primer | 813TCTCATATGTTGGTGACATGTTG | 832CGAAGAAAGCCACAAAGCCAGC |
| Probe | 840TTCCTGAGTCTGGGAGAAGC | 850GCCAAGGCTCAGAACACATCTTC |
| P518 | | |
| Forward primer  | 8GCGCTTACCCCCCTGATCATCTTT  | 877GGACAACATGGAATGGTGCC |
| Reverse primer | 774CTCTGTTCAAGTAGAGGAAAGCCA  | 830GCCAGACCCTTGGCAAGACTT |
| Probe | 732TCTCTGCGCCGCTGGCCC | 841CAGACATGGTACATCGAGGACCA |

**Cloning and Sequence Analysis**—A full-length human cDNA of SP9155 was first identified from Patent WO20001016316. It contained a 1296-base open reading frame encoding 431 amino acid residues (see Fig. 1A). The predicted amino acid sequence revealed that SP9155 was a member of the G protein-coupled receptor (GPCR) superfamily. A negative control, the same amount of empty pCDNA3.1 plasmid was used at 0.25 ng, and eight dilutions were used to generate the standard curve. The human SP9155 standard curve was generated using plasmid dilutions, whereas the P518 data was generated using dilutions of a 350-bp PCR product. The mouse standard curves were generated using plasmids containing the mouse SP9155 and P518 precursor genes, with the dilution ranging from 1 ng to 1 μg. To compare the results obtained using plasmids versus PCR products as standards, all data were averaged and converted to copy number/25 ng of cDNA, thus accounting for the differences in weight between the two.

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NPFF, Orexin, and cholecystokinin (CCK) receptors (Fig. 1B).

Identification of a Novel RF-amide Peptide as a Ligand for SP9155—RF-amides are a well known family of GPCR peptide ligands in which the C terminus is arginine-phenylalanine-amide (17). Recently, novel RF-amide peptides such as hRFP-1, hRFP-2, and KiSS have been identified as ligands for orphan GPCRs (17, 18). To search for novel RF-amide peptides, we used a novel computational approach to search the human genome sequence data base. First, a VTS data base was generated from human genomic sequence using GenScan (20).

Using a novel proprietary pattern-based oligopeptide homology search algorithm (PepPat), the protein VTS data base was searched for genes or exons containing RFG[KR] as a motif, where G[KR] is the peptidase digestion and amidation signal. VTS sequences containing at least one RFG[KR] motif were further analyzed for the presence of a signal peptide and for transmembrane domains. The VTS sequences containing a signal peptide and no transmembrane domain were used to predict a variety of peptides for synthesis and inclusion in our in-house peptide ligand collection for screening against different orphan GPCRs. These orphan GPCRs were transiently transfected into HEK293SFM cells, and screened by FLIPR assay, which measures intracellular calcium mobilization. As a result of the screening, a peptide called P52 was found to specifically activate the orphan GPCR SP9155-transfected HEK293SFM cells (Fig. 3A). The same specificity of P52 was also observed in SP9155-transfected CHO cells (data not shown). However, the EC50 of P52 in both cell lines was only about 250 nM (Tables II and III). Examination of the genomic sequence corresponding to P52 revealed the presence of an uninterrupted open reading frame of 381 bp nucleotides, yielding a 126-residue predicted protein (designated as P518 precursor protein) (Fig. 2). The mouse homolog of the precursor was identified by BLAST search of the GenBank data base. Both human and mouse precursor genes were cloned from cDNA libraries by using the primers designed based on the predicted precursor genes. Analysis of the predicted protein by the PSORT and SignalP programs indicated that the first 22 amino acid residues could serve as a signal peptide, and that no potential transmembrane domains were present, suggesting that the predicted human protein (or cleavage products) could be secreted.

Because the EC50 of P52 was relatively low, a series of peptides were synthesized that included residues extending upstream to other potential peptide processing sites. These longer versions of P52 including P513, P517, and P518 were then tested for activity at SP9155 (Tables II and III). As shown in Table III and Fig. 3, the activities of P51 and P242 for SP9155 are very weak, with EC50 values greater than 1 μM. However, the activity of P552 is comparable with those of P52, P513, and P517 with an EC50 of 600 nM. N-terminal extended peptides from the other RFGR motif of P518 preproprotein (P51, P242, and P552) were also tested for agonist activity for the SP9155 (Fig. 3B). The mouse homolog of the P518 peptide (designated as P550) has similar activity for SP9155 as the P518 peptide, and its EC50 is about 6 nM.
The activation of SP9155 by P518 in the absence of added G proteins, suggests that SP9155 probably couples to Gq. To further determine the effect of G-protein coupling on SP9155, different single chimeric G-proteins including Gq, Gp, G11, G12, and Galpha16, with approximately equivalent efficiencies (data not shown). Moreover, the ability of P518 to mobilize intracellular Ca2+ was then measured by FLIPR (12, 14). It was found that with or without chimeric G proteins the potency of P518 for SP9155 was then measured by FLIPR (12, 14). It was found that with or without chimeric G proteins the potency of P518 for SP9155 is similar (data not shown). Moreover, the ability of P518 to mobilize intracellular Ca2+ via SP9155 was not affected by pertussis toxin treatment (data not shown). Therefore, we conclude that SP9155 is coupled to the Gq signaling pathway.

Expression Profile of P518 Precursor mRNA and SP9155 in Human and Mouse Tissues—The standard curves generated from the plasmids and PCR products indicated that all the Taqman primer and probe sets behaved in the expected manner, with approximately equivalent efficiencies (data not shown). In addition, the 18S and CD4 control probe sets were transcribed with SP9155 transfected 293SFM cells.

TABLE II

| Peptide name | EC50 (nM) |
|--------------|-----------|
| P518         | 7.0 ± 3.0 |
| P550         | 6.0 ± 2.0 |
| P52          | 245 ± 60  |
| P513         | 258 ± 20  |
| P517         | 235 ± 20  |
| P51          | >1500     |
| P242         | >6000     |
| P552         | 607 ± 133 |
| YGGFMRF-amide| >40,000   |

The values represent mean ± S.D., with n = 5. FLIPR assay was performed with SP9155 transfected 293SFM cells.

The activation of SP9155 by P518 in the absence of added G proteins, suggests that SP9155 probably couples to Gq. To further determine the effect of G-protein coupling on SP9155, different single chimeric G-proteins including Gq(12), Gp(16), G11, G12, and Galpha16, with approximately equivalent efficiencies (data not shown). Moreover, the ability of P518 to mobilize intracellular Ca2+ via SP9155 was not affected by pertussis toxin treatment (data not shown). Therefore, we conclude that SP9155 is coupled to the Gq signaling pathway.
transcriptase-PCR reactions with prepared from the indicated tissues were used as templates in reverse quantitative PCR analysis of P518 specific PCR product described under "Experimental Procedures." Results are displayed as copies per 25 ng of cDNA as quantitated using gene-primers as described under "Experimental Procedures." SP9155 in human and mouse tissues. expression of P518 tissues except that mouse tissues are used.

Fig. 4. Expression analysis of peptide P518 precursor and SP9155 in human and mouse tissues. A, quantitative PCR analysis of P518 precursor P518 and SP9155 in human tissues. Total RNA prepared from the indicated tissues were used as templates in reverse transcriptase-PCR reactions with P518 precursor and SP9155-specific primers as described under “Experimental Procedures.” Results are displayed as copies per 25 ng of cDNA as quantitated using genespecific PCR product described under “Experimental Procedures.” B, quantitative PCR analysis of P518 precursor and SP9155 in mouse tissues. The experimental procedures are the same as for human tissues except that mouse tissues are used.

P513, P517, and P518 have a common C-terminal sequence, but they differ in the length of N-terminal sequence. The EC<sub>50</sub> values for peptides P52, P513, and P517 are similar and are around 250 nM, indicating that the N-terminal lysine in P513 and P517 does not have much effect on the potency of peptides. However, the EC<sub>50</sub> for P518 is about 7 nM, which is about 35-fold more potent than P52, P513, and P517, indicating that the N-terminal part of P518 also interacts with SP9155 receptor.

Many RF-amide peptides have been previously identified in lower organisms: in Caenorhabditis elegans, more than 50 distinct RF-amide peptides have been found (21, 22). In such lower organisms these peptides exhibit diverse functions including cardioexitation, control of muscle contraction, and neuromodulation (21, 22). However, in mammals, only a few RF-amide peptides have been reported so far. These include neuropeptide FF (NPFF), neuropeptide AF (NPAF), RFRP (RF-amide-related peptide), all of which have been shown to interact with one or more specific GPCRs (17, 19). Interestingly, the two NPFF receptors are the receptors most homologous to SP9155.

Multiple ligands have been identified for NPFF receptors. In addition to NPFF and NPAF peptides, two RFRP peptides have also been shown to act as ligands for NPFF receptors (17, 19). The RFRP peptides are peptides predicted from a precursor identified through a bioinformatics approach, in a similar manner used to predict the RF-amide peptide P518. A recent survey of GPCR sequences suggested that NPAF and/or NPFF are agonist ligands for SP9155 (23). We found that NPAF, NPFF, and other known peptide ligand GPCR agonists were not functional agonists for SP9155 (data not shown). Additionally, P518 possessed no appreciable agonist activity for other peptide ligand GPCRs, including NPFF and mestastatin.

Previously, Met-Enkephalin-Arg-Phe-amide was shown to activate SP9155, but its EC<sub>50</sub> is greater than 40 μM, and Met-enkephalin-Arg-Phe-amide has not been shown to be an endogenous peptide (patent WO2001016316). In the present study, the high potency of P518 for SP9155 suggests that P518 may be the endogenous ligand for SP9155. There are several lines of evidence to support this possibility. First, the precursor for P518 is derived from genomic sequence, and its physical clones have been obtained from cDNA libraries. Moreover, P518 precursor was shown to be expressed in human tissues under normal physiological conditions. Second, because SP9155 shares high homology to NPFF receptors, and the ligands for NPFF receptors are RF-amide peptides, phylogenetic analysis suggests that the ligand for SP9155 is likely to be an RF-amide peptide. Third, P518 peptide is a potent ligand for SP9155 at low nanomolar and the potency is in the range as expected for an endogenous ligand. Although P518 is most likely the endogenous ligand for SP9155, the current study does not exclude the possibility that there exist additional endogenous peptides similar to or distinctly different from the structure of P518. Confirmation of P518 as the sole or one of the multiple endogenous ligands await studies using natural tissue sources for ligand isolation.

Although a potent ligand for SP9155 has been identified, the biological functions for both the ligand and SP9155 are still not clear. Further studies utilizing animal models such as knockout mice with deficient P518 or SP9155 will be useful in dissecting the functions of the peptide and receptor. Nevertheless, identification of the ligand will provide a research tool to study the functions of SP9155 and allow new agonists and antagonists to be identified by setting up screening assays for SP9155.

REFERENCES
1. Gilman, A. G. (1987) Annu. Rev. Biochem. 56, 615–649
2. Strader, C. D., Fong, T. M., Graziano, M. P., and Tota, M. R. (1995) FASEB J. 9, 745–754
3. Howard, A. D., McAllister, G., Feighner, S. D., Liu, Q., Nargund, R. P., Van der Plough, L. H., and Patchett, A. A. (2001) Trends Pharmacol. Sci. 3, 132–140
4. Civelli, O., Nöthacker, H.-P., Saito, Y., Wang, Z., Lin, S., and Reinscheid, R. K. (2001) Trends Neurosci. 4, 230–237
5. Wilson, S., Bergsma, D. J., Chambers, J. K., Muir, A. I., Fantom, K. G. M., Ellis, C., Murdock, P. R., Herrity, N. C., and Stadel, J. M. (1998) Br. J. Pharmacol. 125, 1387–1392
6. Charmers, J., Ames, R. S., Bergsma, D., Muir, A., Fitzgerald, L. R., Hervieu, G., Dytko, G. M., Foley, J. J., Martin, J., Liu, W. S., Park, J., Ellis, C., Ganguly, S., Kunchar, S., Cluderay, J., Leslie, R., Wilson, S., and Sarau, H. M. (1999) Nature 400, 261–265
7. Shan, L., Qiao, X., Crona J. H., Behan, J., Wang, S., Laz, T., Bayne, M., Gustafson, E. L., Monsma, F. J., Jr., and Hedrick, J. A. (2000) J. Biol. Chem. 275, 39482–39486
8. Zhang, F. L., Luo, L., Gustafson, E., Palmer, K., Qiao, X., Fan, X., Yang, S., Laz, T. M., Bayne, M., and Monsma, F., Jr. (2001) J. Biol. Chem. 276, 8608–8615
9. Reinscheid, R. K., Nothacker, H. P., Bourson, A., Ardati, A., Henningsen, R. A., Bunzow, J. R., Grandy, D. K., Langen, H., Monsma F. J., Jr., and Civelli, O. (1995) Science 270, 792–794
10. Sakurai, T., Amemiya, A., Ishii, M., Matsuoka, I., Chemelli, R. M., Tanaka, H., Williams, S. C., Richardson, J. A., Koizumi, K., Wilson, S., Arch, J. R., Buckingham, R. E., Haynes, A. C., Carr, S. A., Annan, R. S., McNulty, D. E., Liu, W. S., Terret, J. A., Elshourbagy, N. A., Bergsma, D. J., and Yanagisawa, M. (1998) Cell 92, 573–585
11. Zhang, F. L., Luo, L., Gustafson, E., Lachowicz, J., Smith, M., Qiao, X., Liu, Y.-H., Chen, G., Pramanik, B., Laz, T. Z., Palmer, K., Bayne, M., and Monsma, F. J., Jr. (2001) J. Biol. Chem. 276, 8608–8615
12. Hoppelater, G., Jantzen, H. M., Vincent, D., Li, G., England, L., Ramakrishnan, V., Yang, R.-B., Norden, P., Julius, D., and Conley, P. B. (2001) Nature 409, 202–207
13. Conklin, B. R., Farfel, Z., Lustig, K. D., Julius, D., and Bourne, H. R. (1993) Nature 363, 274–276
14. Lee, D. K., Nguyen, T., Lynch, K. R., Cheng, R., Vanti, W. B., Arkhikto, O., Lewis, T., Evans, J. F., George, S. R., and O'Dowd, B. P. (2001) J. Pharmacol. Exp. Ther. 275, 83–91
15. Jiang, Y., Qiao, G., Fang, G., Gustafson, E. L., Laverty, M., Yin, Y., Zhang, Y., Luo, J., Greene, J. R., Bayne, M. L., Hedrick, J., and Murgolo, N. J. et al. (2003) Mamm. Genome 14, 341–349
16. Hinuma, S., Shintani, Y., Fukusumi, S., Iijima, N., Matsumoto, Y., Hosoya, M., Fuji, R., Watanabe, K., Kikuchi, K., Terao, Y., Yano, T., Yamamoto, T., Kawamata, Y., Habata, Y., Asada, M., Kitada, C., Kurokawa, T., Onda, H., Nishimura, O., Tanaka, M., Ibeta, Y., and Fujino, M. (2000) Nat. Cell Biol. 2, 703–708
17. Ohtaki, T., Shintani, Y., Honda, S., Matsumoto, H., Hori, A., Kamehashi, K., Terao, Y., Kuno, K., Takatsu, Y., Masuda, Y., Ishibashi, Y., Watanabe, T., Asada, M., Yamada, T., Suenaga, M., Kitada, C., Usuki, K., Kurokawa, T., Onda, H., Nishimura, O., and Fujino, M. (2001) J. Biol. Chem. 276, 613–617
18. Elshourbagy, N. A., Ames, R. S., Fitzgerald, L. R., Foley, J. J., Chambers, J. K., Szekeres, P. G., Evans, N. A., Schmidt, D. B., Buckley, P. T., Dytko, G. M., Murdock, P. R., Milligan, G., Groarke, D. A., Tan, K. B., Shahin, U., Nathulaqant, P., Wang, D. Y., Wilson, S., Bergsma, D. J., and Sarau, H. M. (2000) J. Biol. Chem. 275, 25965–25971
19. Burge, C., and Karlin, S. (1997) J. Mol. Biol. 268, 78–94
20. Nelson, L. S., Rosoff, M. L., and Li, C. (1998) Science 281, 1686–1690
21. Li, C., Kim, K., and Nelson, L. S. (1999) Brain Res. 848, 26–34
22. Jost, P., and Methner, A. (2002) Genome Biol. 3, 63.1–63.1.6
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