BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **234,** 190–193 (1997)
ARTICLE NO. RC976591

Molecular Cloning and Tissue Expression of a Novel Orphan G Protein-Coupled Receptor from Rat Lung

James A. Bonini,*,†,[1] Spencer M. Anderson,† and Donald F. Steiner*,†
**Howard Hughes Medical Institute and †The Department of Biochemistry and Molecular Biology, The University of Chicago, 5841 S. Maryland Avenue, Chicago, Illinois 60637*

Received April 1, 1997

G protein-coupled receptors transduce the signal of a wide variety of hormones, neurotransmitters, cytokines, and other molecules across the cell membrane to elicit the corresponding response inside the target cells. We describe in this paper the molecular cloning and tissue distribution of a novel rat G protein-coupled receptor, GPR41, with highest homology to the human orphan G protein-coupled receptor DRY12. A lower degree of homology was seen with the receptors for bradykinin, angiotensin, and IL8. The expression of GPR41 appears to be the highest in brain and lung tissues, with lesser expression in heart, skeletal muscle, and kidney, as assayed by northern blotting. No GPR41 message was seen in spleen, liver, or testes. GPR41 failed to bind any of the ligands tested. © 1997 Academic Press

The signal from many hormones, cytokines, and neurotransmitters is transduced across the plasma membrane by serpentine seven-transmembrane receptors belonging to the G protein-coupled receptor (GPCR) superfamily (1). Although these receptors share several amino acid residues and structural features in common, they can be grouped into various subfamilies based on their amino acid sequences and their cognate ligands. In spite of the subclassification of GPCRs, there are many receptors which have been cloned but do not belong to a family or bind any known ligands. There are several of these "orphan" receptors (2,3), and many more continue to be described in the literature and in direct submissions to biological databases such as genbank (4,5).

In this paper, we describe the molecular cloning and expression of a novel rat G protein-coupled receptor, GPR41, using the reverse transcriptase-polymerase chain reaction (RT-PCR) and degenerate oligonucleotide primers. This approach has been successful in cloning many members of the G protein-coupled receptor superfamily due to the conservation of sequences in the transmembrane regions (1,2,6-8). GPR41 has the highest amount of sequence identity to the human IL8-related receptor DRY12 (genbank accession number U58828), sharing 81% amino acid sequence identity.

MATERIALS AND METHODS

Materials. [^{125}I]-islet amyloid polypeptide (IAPP), salmon calcitonin (sCT), [^{125}I]-calcitonin gene related polypeptide (CGRP), and [^{125}I]-IL8 were purchased from Amersham (Arlington Heights, IL). Unlabeled IAPP, CGRP, sCT, and IL8 were purchased from Peninsula Laboratories (Belmont, CA). Manual sequencing reagents were obtained from Amersham (Arlington Heights, IL). All other chemicals and culture media were purchased from GibcoBRL (Gaithersburg, MD). [^{32}P]dATP and [^{32}P]dATP were purchased from NEN (Boston, MA).

Degenerate RT-PCR of rat lung and islet RNA. In an effort to clone a receptor for the islet amyloid polypeptide (IAPP), we performed degenerate PCR on rat lung cDNA. Total RNA was extracted from isolated rat lung tissue by the method of Chomczynski and Sacchi (9). One μg of total RNA was used for reverse transcription with the Superscript preamplification system for first strand cDNA synthesis (Gibco BRL). PCR was carried out on this template using a set of degenerate primers labelled TM3F1 and TM6R, based on the transmembrane domains TM3 and TM6, respectively, of the seven transmembrane G-protein coupled receptors for adrenomedullin (8) and calcitonin gene-related polypeptide (CGRP) (10), since the ligands for these receptors possess the closest structural similarity to IAPP. The nucleotide sequence of TM3F1 was 5′-AGCAT(TC)TTC-TTCCT(CG)AC(CG)TG-3′. The sequence of TM6R was 5′-AC(AG)T-G(AG)TA(TCAG)GG(TCAG)A(AG)CCA(AG)C-3′. The PCR conditions were as follows: 94°C hold for 3 min, followed by 35 cycles of 94°C for 1 min, 49°C for 1.5 min, and 72°C for 1.5 min, followed by a 72°C hold for 4 min. The entire PCR was electrophoresed on a 1% agarose gel, and DNA bands of approximately the expected size (400bp) were cut from the gel and purified using the QIAQUICK gel extraction kit (Chatsworth,CA). Purified DNA was then subcloned into the pCR2.1 vector using the TA cloning kit (Invitrogen, San Diego, CA). Inserts were then sequenced with T7 or M13-20 forward primers using either Sequenase (USB, Cleveland, OH) or an ABI-prizm 370 automated sequencer (Foster City, CA).

Library screening. To obtain full-length clones, a rat lung λgt10 cDNA library CLONTECH, Palo Alto, CA) was screened using a 5′-end [^{32}P]-labeled oligonucleotide primer (5′-GATGAAGAAGACAAG-

[1] Corresponding author. Fax: (773)702-4292. E-mail: jabonini@midway.uchicago.edu.

0006-291X/97 $25.00

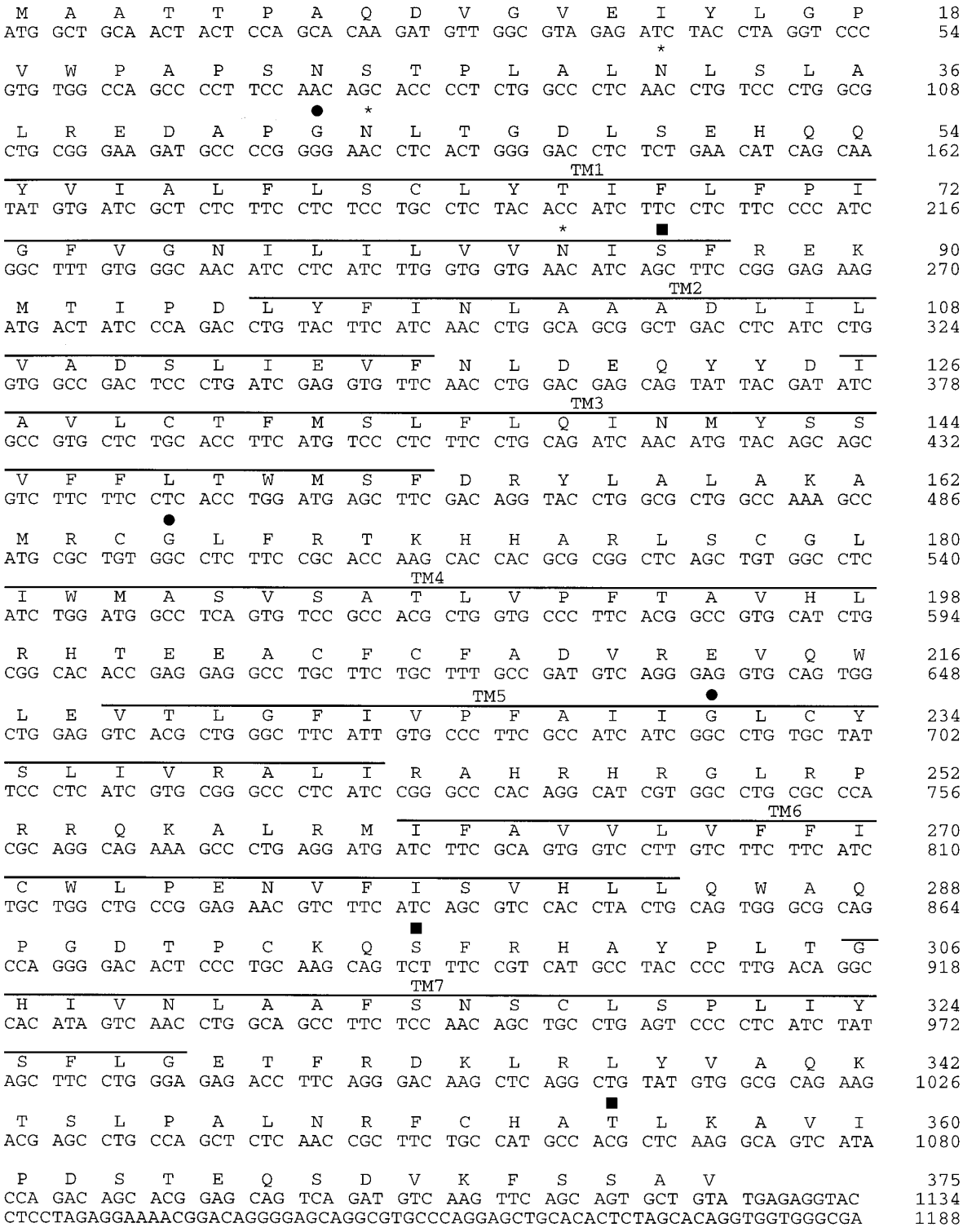

FIG. 1. DNA and predicted amino acid sequence of GPR 41. The seven putative transmembrane domains are labeled TM1-TM7 and indicated by the presence of a line above the corresponding sequence. Potential N-linked glycosylation sites (*), myristylation sites (●), and PKC phosphorylation sites (■) are also indicated above the sequence. The DNA sequence of GPR41 has been deposited into Genbank and has the accession number U92802.

```
GPR41   MAATTPAQDV GVEIYLGPVW PAPSNSTPLA LNLSLALRED APGNLTGDLS  50
DRY12   MDVTSQARGV GLEMYPGTAQ PAAPNTT--- ---------- SP-----ELS  32

GPR41   EHQQYVIALF LSCLYTIFLF PIGFVGNILI LVVNISFREK MTIPDLYFIN 100
DRY12   EHQQYVIGLF LSCLYTIFLF PIGFVGNILI LVVNISFREK MTIPDLYFIN  82

GPR41   LAAADLILVA DSLIEVFNLD EQYYDIAVLC TFMSLFLQIN -MYSSVFFLT 149
DRY12   LAVGDLILVA DSLIEVFNLH ERYYDIAVLC TFMSLFLQVQ HVQAASSFLT 132

GPR41   WMSFDRYLAL AKAMRCGLFR TKHHARLSCG LIWMASVSAT LVPFTAVHLR 199
DRY12   WMSFDRYIAL ARAMRCSLFR TKHHARLSCA LIWMSSVSAT LVPFTAVHLQ 182

GPR41   HTEEACFCFA DVREVQWLEV TLGFIVPFAI IGLCYSLIVR ALIRAHRHRG 249
DRY12   HTDEACFCFA DVREVQWLEV TLGFIVPFAI IGLCYSLIVR VLVRAHRHRG 232

GPR41   LRPRRQKALR MIFAVVLVFF ICWLPENVFI SVHLLQWAQP GDTPCKQSFR 299
DRY12   LRPRRQKALR MILAVVLVFF VCWLPENVFI SVHLLQRTQP GAAPCKQSFR 282

GPR41   HAYPLTGHIV NLAAFSNSCL SPLIYSFLGE TFRDKLRLYV AQKTSLPALN 349
DRY12   HAHPLTGHIV NLAAFSNSCL TPLIYSFLGE TFRDKLRLYI EQKTNLPALN 332

GPR41   RFCHATLKAV IPDSTEQSDV KFSSAV                          375
DRY12   RFCHAVLKVV IPDSTEQSDV RFSSAV                          358
```

FIG. 2. Amino acid alignment of GPR41 and DRY12. Identical amino acid residues are shaded. The percent identity between GPR41 and DRY12 is 81%.

GACCAC-3′) specific for the 400 bp receptor cDNA fragment obtained from the initial degenerate PCR. Out of approximately 1×10^6 phage plated, 3 positive clones were found, one of which contained an insert of approximately 2.0 kb. This clone contained cDNA encoding the entire coding region of the GPR41 cDNA, which was sequenced on both strands using the ABIprizm automated sequencer.

Northern blotting. A rat multiple tissue northern blot was purchased from Clontech (Palo Alto, CA), and probed with nick-translated [^{32}P]dCTP-labelled cDNA probes of the full-length coding sequence of GPR41. Nick translation was performed using the nick translation kit from Amersham (Arlington Heights, IL). Hybridization was performed at 68°C for 1 hour in Expresshybe (Clontech). The blots were washed according to the manufacturer's instructions, and then exposed to X-ray film for 3 days at −80°C with an intensifying screen.

Expression of GPR41 and binding assays with potential ligands. The full-length coding region of GPR41 was directionally subcloned into the HindIII/NotI site of pCDNA 3.1, and transiently transfected into COS7 cells essentially according to the calcium phosphate method of Chen and Okayama (11). Two days post-transfection, cells were washed once in binding media (DMEM containing 0.5% BSA, 20 mM HEPES, pH 7.8) before incubation at 15°C for 4 hours in binding media containing 20 pM ^{125}I-labeled ligand with or without unlabeled competing ligand (100 nM), as previously described (12). Following this incubation, cells were washed two times with one ml of binding buffer, solubilized in 1 ml lysis solution (1% SDS, 100 mM NaCl, 25 mM HEPES, pH 7.8), and counted.

RESULTS AND DISCUSSION

In an effort to clone a receptor for the islet amyloid polypeptide (13), we used PCR on both rat islet and rat lung cDNA. Degenerate PCR with primers based on the third (TM3F1) and sixth (TM6R) transmembrane domains of the seven transmembrane G-protein coupled receptors for adrenomedullin (8) and CGRP (10) yielded several bands on a 1% ethidium bromide stained agarose gel. A band of approximately the expected size (400bp) was excised from the gel, subcloned, and sequencing of the cDNAs in this band yielded several species of sequences. One of the cDNAs, named p2441, was closest in sequence to the human orphan receptor named the "IL8-related receptor," or DRY12 (accession# U58828), an unpublished GPCR cloned from HepG2 cells and directly submitted to Genbank by McCoy, R.L. and Perlmutter, D.H. of Washington University School of Medicine, St. Louis, MO. Subsequent screening of a rat lung λgt10 library using an internal oligonucleotide specific for p2441 resulted in the cloning of the whole coding region of the cDNA, which we called GPR41. In-frame translation of the GPR41 cDNA sequence indicated that the amino acid sequence was also likely to be a seven transmembrane-containing GPCR, and that it possessed an 81% amino acid identity with DRY12. The DNA sequence of GPR41 has been deposited into Genbank, and has the accession number U92802. The sequence and predicted trans-

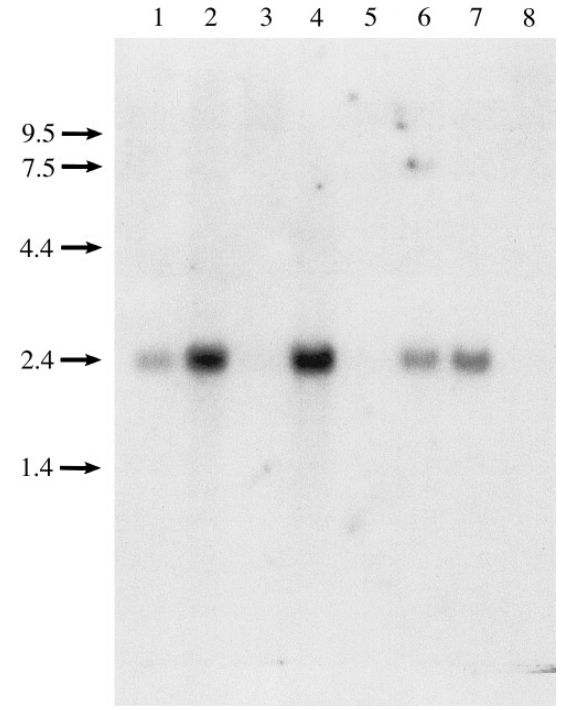

FIG. 3. Multiple tissue Northern blot of the expression of GPR41 in a variety of rat tissues. A rat multiple tissue northern blot was hybridized with a full-length GPR41 cDNA probe. Each lane contains 2 μg poly A^+ RNA from rat heart (lane 1), brain (lane), spleen (lane 3), lung (lane 4), liver (lane 5), skeletal muscle (lane 6), kidney (lane 7), and testes (lane 8).

lation are shown in Fig. 1. An alignment of GPR41 with DRY12, demonstrating the homology of the two amino acid sequences, is shown in Fig. 2.

Although GPR41 and DRY12 are clearly G protein-coupled receptors, the greatest degree of identity to any known GPCR is only 25-26%. Receptors with this degree of identity to GPR41 and DRY12 include the receptors for bradykinin (accession# L26173), IL8 (P25024 and P25025), angiotensin I and II (P25095 and P29089), and the orphan receptors V28 (U20350) and GPRCY6 (U45983). When GPR41 was subcloned into the mammalian expression vector pCDNA 3.1 and expressed in COS7 cells, it failed to bind to IAPP, salmon calcitonin, calcitonin gene-related polypeptide, or IL8 (data not shown). Although it is possible that the lack of measurable binding of these ligands could be the result of poor or defective expression of this receptor construct, both strands of the pCDNA3.1/GPR41 construct were sequenced twice to confirm proper orientation and sequence fidelity.

Northern blot analysis indicated that GPR41 is expressed in several tissues, as shown in Fig. 3. Highest mRNA levels were seen in brain and lung tissues, with lower mRNA abundance in heart, skeletal muscle, and kidney. No signal was seen in the spleen, liver, or testes. This expression pattern is quite different from the expression of DRY12 (D. Perlmutter, personal communication), suggesting that GPR41 may represent a closely related but entirely different receptor from DRY12, rather than a rat homolog. However, this does not rule out the possibility that it is indeed a rat homolog of DRY12, and that it could have a different range of tissue-specific expression in rat as compared to human.

In summary, we report the cloning and tissue expression of a novel G protein-coupled receptor from rat lung, which is most closely related to the human orphan receptor DRY12. It is likely that GPR41 and DRY12 represent a new subfamily of G protein-coupled receptors, given their low degree of identity with any of the known receptors. The molecular cloning of GPR41 and the subsequent characterization of this receptor will hopefully further advance our understanding of the complex signals utilizing G protein-coupled receptors.

ACKNOWLEDGMENTS

The authors thank Paul Gardner and Jeff Stein for outstanding oligonucleotide synthesis.

REFERENCES

1. Probst, W. C., Snyder, L. A., Schuster, D. I., Brosius, J., and Sealfon, S. C. (1992) *DNA Cell Biol.* **11,** 1–20.
2. Heiber, M., Docherty, J. M., Shah, G., Nguyen, T., Cheng, R., Heng, H. H. Q., Marchese, A., Tsui, L-C., Shi, X., George, S. R., and O'Dowd, B. F. (1995) *DNA Cell Biol.* **14,** 25–35.
3. O'Dowd, B. F., Scheidler, M. A., Nguyen, T., Cheng, R., Rasmussen, J. S., Marchese, A., Zastawny, R., Heng, H. H. Q., Tsui, L.-C., Shi, X., Asa, S., Puy, L., and George, S. (1995) *Genomics* **28,** 84–91.
4. Gish, W., and States, D. J. (1993) *Nat. Genet.* **3,** 266–272.
5. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) *J. Mol. Biol.* **215,** 403–410.
6. Raport, C. J., Schweickart, V. L., Eddy, R. L., Jr., Shows, T. B., and Gray, P. W. (1995) *Gene* **163,** 295–299.
7. Power, C. A., Meyer, A., Nemeth, K., Bacon, K. B., Hoogewerf, A. J., Proudfoot, A. E. I., and Wells, T. N. C. (1995) *J. Biol. Chem.* **270,** 19495–19500.
8. Kapas, S., Catt, K. J., and Clark, A. J. L. (1995) *J. Biol. Chem.* **270,** 25344–25347.
9. Chomczynski, P., and Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–159.
10. Kapas, S., and Clark, A. J. L. (1995) *Biochem. Biophys. Res. Commun.* **217,** 832–838.
11. Chen, C., and Okayama, H. (1987) *Mol. Cell. Biol.* **7,** 2745–2752.
12. Kristensen, C., Andersen, A. S., Hach, M., Wiberg, F. C., Schaffer, L., and Kjeldsen, T. (1995) *Biochem J.* **305,** 981–986.
13. Poyner, D. (1995) *TiPS* **16,** 424–428.