Volume 318, number 1, 17–22 FEBS 12121 February 1993

Molecular cloning of a novel rat G-protein-coupled receptor gene expressed prominently in lung, adrenal, and liver

Jeffrey K. Harrison[a], Cynthia M. Barber[a] and Kevin R. Lynch[a,b]

Departments of [a]Pharmacology and [b]Biochemistry, University of Virginia School of Medicine, Charlottesville, VA 22908, USA

Received 18 December 1992

A gene and cDNA (named G10d) encoding a novel member of the G-protein-coupled receptor (GCR) superfamily has been isolated from a rat genomic library and a rat liver cDNA library by tandom polymerase chain reaction (PCR) amplification and hybridization screening. Comparison of the sequence of the encoded protein to other members of the rhodopsin family of the GCR superfamily suggests that this new member is a peptide receptor. The G10d gene is present as a single copy in the rat genome and its translational open reading frame is intronless. Northern analysis demonstrates a wide and uneven distribution of G10d mRNA in the adult rat, with the highest mRNA abundance in lung, liver and adrenal gland extracts.

G-protein-coupled receptor; Putative G-protein-coupled receptor; Orphan receptor; Rhodopsin family; GCR

1. INTRODUCTION

The study of cell surface G-protein-coupled receptors (GCRs) has been facilitated greatly by molecular cloning techniques. From these studies it has become apparent that there exists a large gene superfamily encoding receptors for a wide variety of hormones and neurotransmitters. The primary structure of these receptors are characterized by the presence of seven stretches of hydrophobic amino acids supposedly capable of forming transmembrane (TM) domains. These regions are separated by variable stretches of hydrophilic amino acids predicted to form intra- and extracellular regions. Most of the conserved amino acids are found within the putative transmembrane domains, particularly TM3 and TM6. This conservation has provided a basis from which additional members of this gene family have been isolated [1–10]. Herein we describe the molecular cloning of a novel member of the GCR family. We report the sequence of this putative receptor and the tissue distribution of its mRNA. Although we have not yet identified the ligand for the G10d receptor, we predict that it recognizes a peptide ligand.

2. MATERIALS AND METHODS

2.1. *Polymerase chain reaction amplification, genomic library screening and DNA sequencing*

Two degenerate oligonucleotides (5′-CTCACGTGYMTSAGCPTSGAYCGCTA-3′ and 5′-TGGTAGGGGASCCAGSAMAMGAPPAA-3′; Y = T or C; M = A or C; S = G or C; and P = A or G) were synthesized and used to amplify from rat liver or brainstem cDNA, by PCR, fragments encoding novel members of the rhodopsin GCR family. The oligonucleotide sequences were based primarily on the sequences of TM3 and TM6 from the angiotensin (AT1) receptor [11] and RDC-1 [1] cDNAs. cDNA synthesized from rat liver and rat brainstem poly(A)$^+$ RNA served as the template in the reactions. The conditions of the PCR were as follows: 94°C, 1 min (denaturation); 40°C, 2 min (annealing); 72°C, 3 min (primer extension); 30 cycles. Products of the reaction were subjected to the action of the Klenow fragment of DNA *Pol*I (in the presence of dNTPs), and then treated further with T4 polynucleotide kinase (in the presence of ATP). The products were then subcloned into the *Sma*I site of pGEM7Zf(+) (Promega) for sequence analysis (Sanger dideoxy chain termination, Sequenase kit, IBI).

A 360 bp fragment (jh10) predicted by sequence analysis to be a portion of a member of the GCR family was labelled (random primer method) to a specific activity of approximately 1×10^9 dpm/μg and used to screen 7.5×10^5 recombinants of an *Hae*III partialled Sprague–Dawley rat genomic DNA library (λCharon 4a, gift of T. Sargent, [12]). The bacteriophage responsible for one signal was plaque purified (λG10d). Phage DNA was isolated by standard procedures and subjected to restriction enzyme digestion. A 3 kb *Hin*dIII fragment (G10d) hybridizing to the jh10 DNA was subcloned to the same site in pGEM7Zf(+) and subjected to sequence analysis.

Two oligonucleotides (5′-CAGACACAAGCTTGCCCTCATGTC-3′ and 5′-TGTCTCGAGTCTACCTTAGCTGGCTAT-3′) were synthesized and used to amplify by PCR (rat liver cDNA as template) the entire coding region of G10d. This PCR product was restriction digested with *Hin*dIII and *Xho*I and subcloned to the *Hin*dIII/*Xho*I sites of pCDM8 (Invitrogen). The resultant insert DNA was subjected to sequence analysis. The plasmid was also used to transfect *COS-1* cells as described previously [13]. Plasmid was linearized with *Xho*I, subjected to in vitro transcription, and injected into stage V or VI *Xenopus laevis* oocytes. Oocyte responses (I_{ClCa_1}) were recorded as described previously [14].

2.2. *Hybridization analysis of rat genomic DNA and RNA*

Sprague–Dawley rat genomic DNA was isolated by standard procedures [15]. Restriction digested rat genomic DNA (15 μg) was subjected to Southern blot analysis. A 1.2 kb *Hin*dIII/*Xho*I DNA frag-

Correspondence address: K.R. Lynch, Department of Pharmacology, Box 448, University of Virginia School of Medicine, 1300 Jefferson Park Avenue, Charlottesville, VA 22908, USA. Fax: (1) (804) 982-3878.

ment containing the entire coding region of G10d was labelled by the random primer method ($1–2 \times 10^9$ dpm/μg) and used as a hybridization probe using the method of Church and Gilbert [16]. Final wash temperature was 65°C. Exposure to Kodak XAR film was 2 days at −70°C with intensifying screen.

Total cellular RNA from a variety of rat tissues was isolated by the guanidine isothionate/CsCl method [15]. Poly(A)$^+$-selected RNA was prepared by oligo(dT) cellulose chromatography. RNA (4–5 μg) was electrophoresed through denaturing 1.2% agarose and subjected to Northern analysis [16] using the same 1.2 kb DNA fragment described above. Final wash temperature was 65°C. Exposure to Kodak XAR film was for 5 days at −70°C with intensifying screen.

3. RESULTS AND DISCUSSION

PCR products were generated from either rat brainstem or liver cDNAs using degenerate oligonucleotides corresponding to sequences found in TM3 and TM6 of the angiotensin (AT1) receptor and RDC-1 cDNAs. Sequence analysis suggested that one set of clones (jh10) contained sequences encoding a fragment of a novel member of the rhodopsin branch of the GCR superfamily. The predicted polypeptide sequence was similar to

```
     TAGCTGGCCCTCATGTCAGTCATACCCAGCTCCAGGCCGGTCTCCACCTTGGCACCTGAC   60
     *          M  S  V  I  P  S  S  R  P  V  S  T  L  A  P  D

     AATGATTTTAGAGAGATCCACAACTGGACAGAGCTGCTCCATCTCTTCAACCAAACCTTT  120
 17  N  D  F  R  E  I  H  N  W  T  E  L  L  H  L  F  N  Q  T  F

     TCTGATTGCCACATGGAACTCAATGAGAACACCAAACAAGTGGTCCTCTTTGTCTTCTAC  180
 37  S  D  C  H  M  E  L  N  E  N  T  K  Q  V  V  L  F  V  F  Y

     CTGGCCATCTTTGTGGTAGGGTTAGTGGAGAACGTCCTGGTGATATGTGTCAACTGCCGC  240
 57  L  A  I  F  V  V  G  L  V  E  N  V  L  V  I  C  V  N  C  R

     CGCTCAGGCCGGGTGGGGATGCTGAACTTATACATCCTCAACATGGCCGTCGCGGACCTG  300
 77  R  S  G  R  V  G  M  L  N  L  Y  I  L  N  M  A  V  A  D  L

     GGCATCATCCTGTCTCTGCCTGTTTGGATGCTGGAGGTCATGCTGGAGTACACCTGGCTC  360
 97  G  I  I  L  S  L  P  V  W  M  L  E  V  M  L  E  Y  T  W  L

     TGGGGCAGCTTCTCCTGTCGCTTCATTCATTATTTCTACCTTGCCAACATGTACAGCAGC  420
117  W  G  S  F  S  C  R  F  I  H  Y  F  Y  L  A  N  M  Y  S  S

     ATCTTCTTCCTCACCTGCCTCAGCATTGACCGCTACGTCACCCTCACCAATACCTCTCCC  480
137  I  F  F  L  T  C  L  S  I  D  R  Y  V  T  L  T  N  T  S  P

     TCCTGGCAGCGCCACCAGCACCGAATACGGAGGGCCGTGTGCGCAGGCGTCTGGGTCCTC  540
157  S  W  Q  R  H  Q  H  R  I  R  R  A  V  C  A  G  V  W  V  L

     TCCGCCATCATCCCACTGCCTGAGGTGGTACATATCCAGCTGCTGGATGGCTCCGAGCCC  600
177  S  A  I  I  P  L  P  E  V  V  H  I  Q  L  L  D  G  S  E  P

     ATGTGCCTCTTCCTAGCACCTTTTGAAACGTACAGCGCCTGGGCCCTGGCAGTGGCCCTG  660
197  M  C  L  F  L  A  P  F  E  T  Y  S  A  W  A  L  A  V  A  L

     TCGGCTACCATCCTGGGCTTCCTACTGCCTTTTCCTCTCATCGCAGTGTTTAATATCCTG  720
217  S  A  T  I  L  G  F  L  L  P  F  P  L  I  A  V  F  N  I  L

     TCAGCCTGCCGGCTTCGGAGGCAAGGGCAGACAGAGAGCAGGCGCCACTGTCTGTTGATG  780
237  S  A  C  R  L  R  R  Q  G  Q  T  E  S  R  R  H  C  L  L  M

     TGGGCTTACATAGTTGTCTTTGCCATCTGCTGGCTGCCCTACCACGTGACTATGCTGCTG  840
257  W  A  Y  I  V  V  F  A  I  C  W  L  P  Y  H  V  T  M  L  L

     CTCACTCTGCACACAACCCACATCTTCCTTCACTGCAACCTGGTTAACTTCCTCTACTTC  900
277  L  T  L  H  T  T  H  I  F  L  H  C  N  L  V  N  F  L  Y  F

     TTCTACGAAATCATTGACTGCTTCTCCATGCTACACTGTGTCGCCAACCCCATCCTCTAC  960
297  F  Y  E  I  I  D  C  F  S  M  L  H  C  V  A  N  P  I  L  Y

     AACTTTCTCAGCCCGAGCTTCCGGGGCCGACTGCTGAGCCTTGTGGTCCGTTACCTTCCC 1020
317  N  F  L  S  P  S  F  R  G  R  L  L  S  L  V  V  R  Y  L  P

     AAGGAGCAGGCCAGGGCAGCAGGCGGTCGAGCCTCCTCTTCATCTTCCACCCAGCACTCC 1080
337  K  E  Q  A  R  A  A  G  G  R  A  S  S  S  S  S  T  Q  H  S

     ATCATCATTACCAAAGAGGGCAGCCTGCCGCTGCAGCGGATCTGCACACCCACGCCATCA 1140
357  I  I  I  T  K  E  G  S  L  P  L  Q  R  I  C  T  P  T  P  S

     GAAACGTGCAGGCCTCCTCTCTGCCTCCGAACACCTCACCTACACTCTGCAATTCCATAG 1200
377  E  T  C  R  P  P  L  C  L  R  T  P  H  L  H  S  A  I  P  *  395
```

Fig. 1. The amino acid sequence (single-letter code) of the G10d protein as conceptualized from the G10d genomic and cDNA clones. The putative transmembrane-spanning domains are underlined. Asn residues in the context of *N*-linked glycosylation consensus sequences are boldfaced. The sequence of G10d has been deposited to the Genbank database (Accession: L09249).

```
          1                                                          60
RAT G10d  .........M SVIPSSRPVS TLAPDNDFRE IHNWTELLHL FNQT...FSD CHMELNENTK
DOG RDC1  .......... .......... MDLHLFDYAE PGNFSDISWP CNSSDCIVVD TVLCPNMPNK
RAT AT1   .......... .......... .......... ......MALN SSAE.DGIKR IQDDCPKAGR
RAT BK2   MDTRSSLCPK TQAVVAVFWG PGCHLSTCIE MFNITTQALG SAHN.GTFSE V..NCPDTEW
HUM IL8   .......... .......... ...MESDSFE DFWKGEDLSN YSYS.STLPP FLLDAAPCEP
RAT SOM   .......... .......... ....MELTSE QFNGSQVWIP SPFDLNGSLG PSNGSNQTEP

          61                                                         120
RAT G10d  QVVLF...VF ...YLAIFVV GLVENVLVIC VNCRRSGRVG MLNLYILNMA VADLGIILSL
DOG RDC1  SVLLY...TL SFIYIFIFVI GMIANSVVVW VNIQAKTTGY DTHCYILNLA IADLWVVVTI
RAT AT1   HSYIFV..MI PTLYSIIFVV GIFGNSLVVI VIYFYMKLKT VASVFLLNLA LADLCFLLTC
RAT BK2   WSWLNA..IQ APFLWVLFLL AALENIFVLS VFCLHKTNCT VAEIYLGNLA GADLILACGL
HUM IL8   ESLEINKYFV VIIYALVFLL SLLGNSLVML VILYSRVGRS VTDVYLLNLA LADLLFALTL
RAT SOM   YYDMTSNAVL TFIYFVVCVV GLCGNTLVIY VILRYAKMKT ITNIYILNLA IADELFMLGL

          121                                                        180
RAT G10d  PVWMLEVMLE YTWLWGSFSC RFIHYFYLAN MYSSIFFLTC LSIDRYVTLT NTSPSWQRHQ
DOG RDC1  PVWVVSLVQH NQWPMGELTC KITHLIFSIN LFGSIFFLTC MSVDRYLSIT YFASTSSRRK
RAT AT1   PLWAVYTAME YRWPFGNHLC KIASASVTFN LYASVFLLTL LSIDRYLAIV HPMKSRLRRT
RAT BK2   PFWAITIANN FDWLFGEVLC RVVNTMIYMN LYSSICFLML VSIDRYLALV KTMSMGRMRG
HUM IL8   PIWAASKVNG ..WIFGTFLC KVVSLLKEVN FYSGILLLAC ISVDRYLAIV HATR.TLTQK
RAT SOM   PFLAMQVAL. VHWPFGKAIC RVVMTVDGIN QFTSIFCLTV MSIDRYLAVV HPIKSAKWRR

          181                                                        240
RAT G10d  HRIRRAVCAG VWVLSAIIPL PEVVH..IQL LDGS...EPM CLFLAPFETY SAWALAVALS
DOG RDC1  KVVRRAVCVL VWLLAFCVSL PDTYY..LKT VTSASNNETY CRSFYPEHSV KEWLISMELV
RAT AT1   MLVGKVTCII IWLMAGLASL PAVIHRNV.. YFIENTNITV CAFHYESRNS TLPIGLG.LT
RAT BK2   VRWAKLYSLV IWSCTLLLSS PMLVFRTMKD YREEGHNVTA CVIVYPSRSW EVFTNML.L.
HUM IL8   RYLVKFICLS IWGLSLLLAL PVLLFRRT.. V.YSSNVSPA CYEDM.GNNT ANWRMLLRIL
RAT SOM   PRTAKMINVA VWGVSLLVIL PIMIYAGL.. .RSNQWGRSS CTINWPGESG AWYTGFI.IY

          241                                                        300
RAT G10d  ATILGFLLPF PLIAVFNILS ACRLRRQGQT ESRRHCL... .....LMWAY IVVFAICWLP
DOG RDC1  SVVLGFAIPF CVIAVFYCLL ARAISASSDQ EKQSSRK... .....IIFSY VVVFLVCWLP
RAT AT1   KNILGFLFPF LIILTSYTLI WKALK.KAYE IQKNKPRNDD ..IFRIIMAI VLFFFFSWVP
RAT BK2   .NLVGFLLPL SII.TFCTVR IMQVL.RNNE MKKFKEVQTE KKATVLVLAV LGLFVLCWFP
HUM IL8   PQSFGFIVPL LIMLFCYGFT LRTLF.KAHM GQKHR..... ..AMRVIFAV VLIFLLCWLP
RAT SOM   AFILGFLVPL TIICLCYLFI IIKVKSSGIR VGSSKRKKSE KKVTRMVSIV VAVFIFCWLP

          301                                                        360
RAT G10d  YHVTMLLLTL HTTH.IFLHC NLVNFLYFFY EIIDCFSMLH CVANPILYNF LSPSFRGRLL
DOG RDC1  YHVVVLLDIF SILHYIPFTC QLENFLFTAL HVTQCLSLVH CCVNPVLYSF INRNYRYELM
RAT AT1   HQIFTFLDVL IQLGVIHD.C KISDIVDTAM PITICIAYFN NCLNPLFYGF LGKKFKKYFL
RAT BK2   FQISTFLDTL LRLGVLSG.C WNERAVDIVT QISSYVAYSN SCLNPLVYVI VGKRFRKKSR
HUM IL8   YNLVLLADTL MRTQVIQETC ERRNHIDRAL DATEILGILH SCLNPLIYAF IGQKFR...H
RAT SOM   FYI..... FNVSSVSVAI SPTPALKGMF DFVVILTYAN SCANPILYAF LSDNFKKSFQ

          361                                                        420
RAT G10d  SLVVRYLPKE QARAAGGRAS SSSSTQHSII ITKEGSLPLQ RICTPTPSET CRPPLCLRTP
DOG RDC1  KAFIFKYSAK TGLTKLIDAS RVSETEYSAL EQNAK..... .......... ..........
RAT AT1   QLLKYIPPKA KS.......H SSLSTKMSTL SY.RPSDNMS SSAKKPASCF EVE.......
RAT BK2   EVYQAICRKG GCMGESVQME NSMGTLRTSI SVDRQIHKLQ DWAGNKQ... ..........
HUM IL8   GLLKILAIHG LISKDSLPKD SRPSFVGSSS GHTSTTL... .......... ..........
RAT SOM   NVLCLVKVSG AEDGERSDSK QDKSRLNETT ETQRTLLNGD LQTSI..... ..........

          421
RAT G10d  HLHSAIP
```

Fig. 2. Alignment of the orphan receptors rat G10d and dog RDC-1, with the rat angiotensin II (AT_1), rat B_2-bradykinin, human interleukin (IL-8), and rat somatostatin receptors. Alignment was achieved using the PILEUP algorithm of the GCG package [23]. Gaps (.) were introduced to maximize the alignment.

corresponding sequences of other members of the family (i.e. TM4 and TM5). This DNA fragment was used subsequently to screen (under conditions of high stringency) 7.5×10^5 recombinants of a rat genomic DNA library. A single clone was isolated and analyzed further. Sequence analysis of a 3 kbp *Hin*dIII fragment (G10d) identified a translational open reading frame (orf) encoding a protein of 395 amino acids. The sequence of the PCR product (jh10) was entirely contained within this orf. Fig. 1 shows the conceptualized amino acid sequence and the underlying nucleotide sequence. A termination codon is found in front of, and in frame with, the putative initiator methionine. In addition, the ATG encoding this methionine is embedded

within a consensus sequence for optimal translational initiation. Hydropathy analysis indicated the presence of seven stretches of hydrophobic amino acids predicted to form the transmembrane domains. Structural features of G10d shared by other members of the gene family include the presence of *N*-linked glycosylation consensus sequences (Asn-Xaa-Ser/Thr: Asn-24 and Asn-33) near the N-terminus, cysteine residues in the putative exofacial loops, as well as the presence of a number of serine or threonine residues in the C-terminal tail that might serve as potential sites of phosphorylation. The only unusual feature of the G10d sequence is that a highly conserved leucine found in TM2 is replaced by a methionine residue.

A search (FASTA [17]) of the Genbank (release #74) database reveals that the sequence of G10d is most similar to receptors for the peptides angiotensin II [11], bradykinin [18], somatostatin [19], interleukin-8 [20], fMLF [21], and C5a [22]. However, the highest degree of similarity was found between G10d and the orphan (i.e. ligand unknown) receptor, RDC-1 (33% identity). Fig. 2 shows a multiple alignment of the orphan receptors, G10d and RDC-1, with receptors for angiotensin II (AT_1), bradykinin, interleukin-8, and somatostatin. Most of the similarity between these receptors lies within the putative transmembrane domains. Very recently, we became aware of a Genbank release (accession number L04672, 3 Dec. 1992) indicating that Eva and Sprengel have identified what is probably the same orf.

Southern blot analysis of restriction-digested rat genomic DNA using a radiolabelled G10d DNA fragment containing the entire coding region showed hybridization to 9 kbp *Bam*HI, 5.5 kbp *Eco*RI, and 3 kbp *Hin*dIII genomic fragments (Fig. 3). The *Hin*dIII fragment is consistent with that obtained in the isolation of the genomic clone (λG10d). Amplification (by PCR) of the coding region of G10d (using cDNA synthesized from rat liver poly(A)$^+$ RNA) and subsequent sequencing revealed that the cDNA and genomic sequences were entirely contiguous. Taken together, these data suggest that the rat genome contains a single copy, intronless (as regards the translational orf) gene encoding the G10d protein.

Northern blot analyses were performed to determine the tissue distribution of G10d mRNA (Fig. 4). G10d mRNA accumulation was widely and unevenly distributed in the adult rat. Hybridization was most prominent in extracts of lung, liver, and adrenal. G10d mRNA accumulation was detected also in kidney, aorta, heart, spinal cord, gut, and testes. Very little hybridization was detected in extracts of discrete regions of the rat brain,

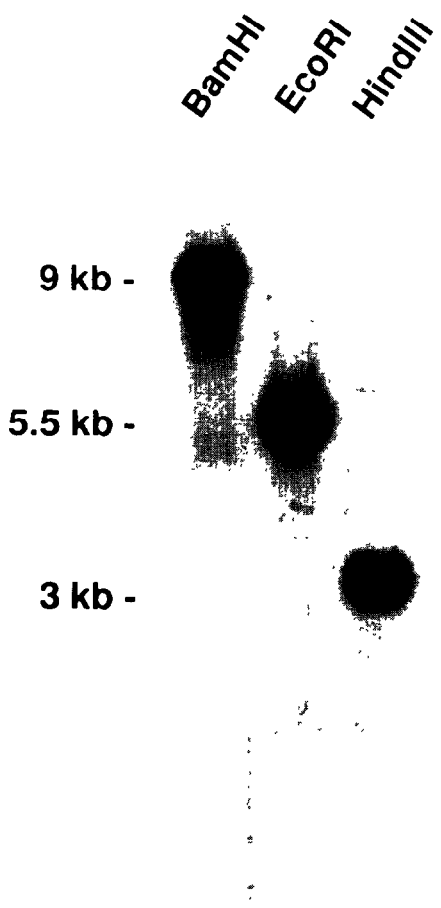


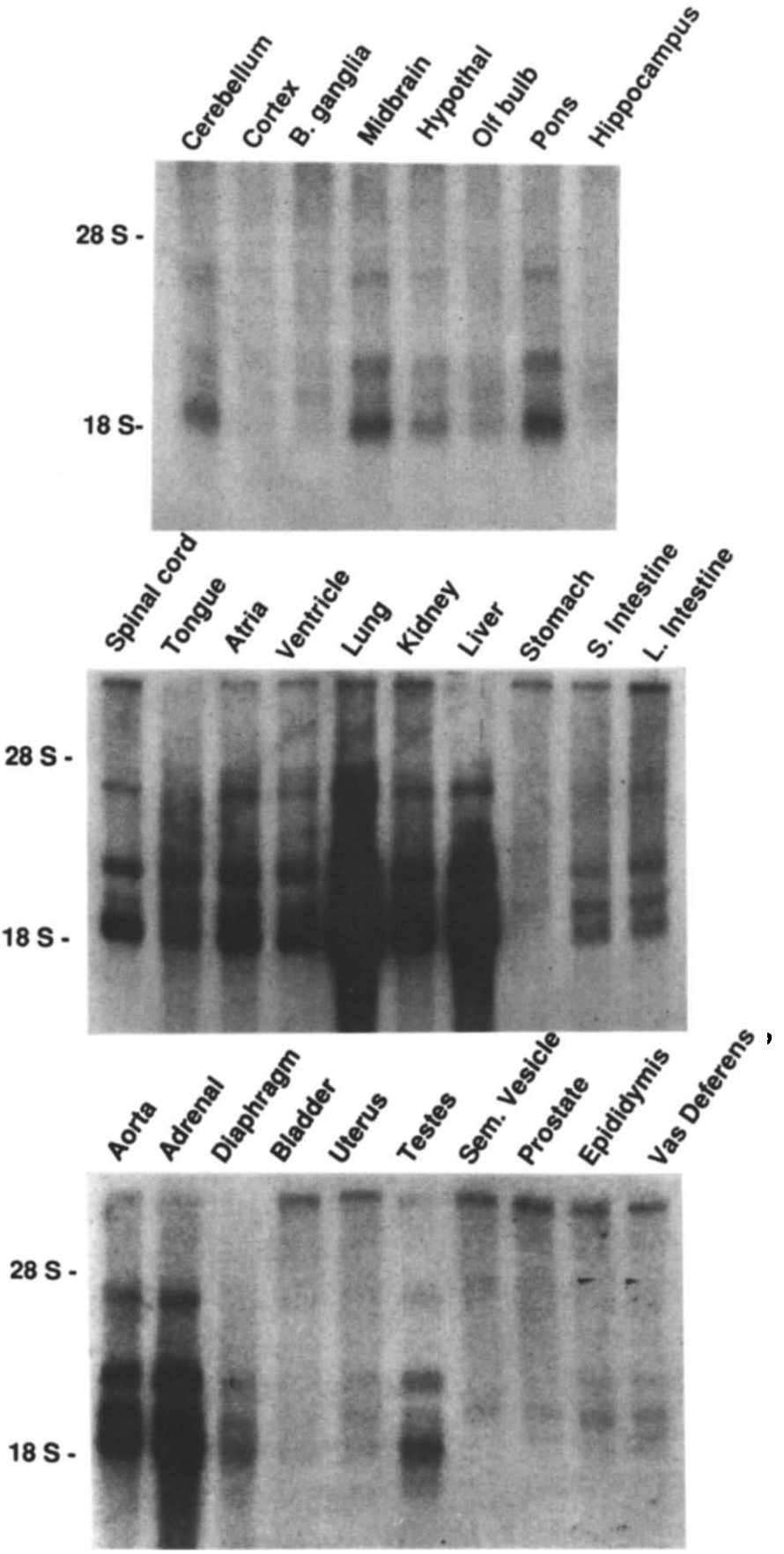
Fig. 3. Southern blot analysis of restriction endonuclease-digested rat genomic DNA. Sprague–Dawley rat genomic DNA (15 μg) was digested completely with *Bam*HI, *Eco*RI and *Hin*dIII and then subjected to Southern blot analysis as described in section 2. The individual band sizes are indicated and were estimated by comparison to the BRL 1-kbp ladder.

although some signal was detected in midbrain and pons. In the tissue extracts where G10d mRNA was detected, multiple size transcripts (2.2, 2.4, 2.8, 4.3, and 7.5 kb) were present. The hybridization and wash conditions were identical for the Northern and Southern analysis, and therefore it is likely that the RNA species detected arise from a single loci of the rat genome. Since the G10d gene is without introns within the translational orf, it is likely also that the differences in the sizes of the mRNAs detected is due to multiple polyadenylation sites along the G10d gene.

To characterize the nature of the G10d protein, the coding region cDNA was transiently expressed in *COS-1* cells. Membranes from transfected cells were prepared and a number of radiolabelled ligands were tested for

→

Fig. 4. G10d mRNA accumulation throughout the rat. Poly(A)$^+$-selected RNA from rat tissue (4 μg from brain extracts; 5 μg from all others) was subjected to Northern analysis as described in section 2. All RNA was from adult animals, except tongue RNA was from 1-day-old animals. The positions of the migration of 18 S and 28 S ribosomal RNA are shown.


Cerebellum
Cortex
B. ganglia
Midbrain
Hypothal
Olf bulb
Pons
Hippocampus
28 S -
18 S-
Spinal cord
Tongue
Atria
Ventricle
Lung
Kidney
Liver
Stomach
S. Intestine
L. Intestine
28 S -
18 S -
Aorta
Adrenal
Diaphragm
Bladder
Uterus
Testes
Sem. Vesicle
Prostate
Epididymis
Vas Deferens
28 S -
18 S -

specific binding. No significant difference in binding to transfected membranes compared to untransfected membranes was detected with [^{125}I]angiotensin II, [^{3}H]Dup 753 (losartan, an angiotensin II receptor antagonist), [^{3}H]naloxone, [^{3}H]leukotriene C4, [^{3}H]leukotriene D4, [^{3}H]bradykinin, [^{125}I]galanin, or [^{3}H]WEB2086 (a platelet-activating factor receptor antagonist). In addition, *Xenopus laevis* oocytes injected with in vitro transcribed (m)RNA prepared from G10d did not respond (Ca^{2+}-activated Cl^- current) to application of angiotensin II, bradykinin, IL-8, MCAF, MIP-1α, MIP-1β, RANTES, TNF-α, ATP, leukotriene C4 or leukotriene D4.

In summary, we have isolated the gene and cDNA for a novel member of the GCR superfamily. The encoded protein is most similar to a set of receptors that are activated by peptide ligands. The mRNA for G10d accumulates in a wide variety of peripheral tissues in the adult rat with predominate expression in lung, liver, and adrenal gland. While we have failed to date to determine the nature of the ligand that binds to, and activates, this putative receptor, we speculate that the G10d protein is a receptor for a peptide ligand.

Acknowledgements: The authors wish to thank the following individuals: (i) Dr. Thomas Sargent (NIH) for his gift of the rat genomic library; (ii) Dr. F.L. Kolakowski (Mass. General Hosp.) for alerting us to the release of the G10d sequence deposited with the Genbank by Eva and Sprengel (Accession #L04672, DNA Cell Biol. (1993), in press); and (iii) Dr. M.E. Durieux and M.N. Salafranca for their help with the oocyte expression and recording. J.K.H. was supported by an NRSA post-doctoral fellowship (F32 HL08223). K.R.L. is supported by National Institutes of Health Grant (P01 HL19242) and is also an Established Investigator of the American Heart Association.

REFERENCES

[1] Libert, F., Parmentier, M., Lefort, A., Dinsart, C., Van Sande, J., Maenhaut, C., Simons, M.-J., Dumont, J.E. and Vassart, G. (1989) Science 244, 569–572.
[2] Parmentier, M., Libert, F., Maenhaut, C., Lefort, A., Gerard, C., Perret, J., Van Sande, J., Dumont, J.E. and Vassart, G. (1989) Science 246, 1620–1622.
[3] Monsma, F.J., Mahan, L.C., McVittie, L D., Gerfen, C.R. and Sibley, D.R. (1990) Proc. Natl. Acad. Sci. USA 87, 6723–6727.
[4] Zhou, Q.-Y., Grandy, D.K., Thambi, L., Kushner, J.K., Van Tol, H.H.M., Cone, R., Pribnow, D., Salon, J., Bunzow, J R. and Civelli, O. (1990) Nature 347, 76–80.
[5] Sunahara, R.K., Niznik, H.B., Weiner, D.M., Stormann, T.M , Brann, M.R., Kennedy, J.L., Gleernter, J.E., Rozmahel, R., Yang, Y., Israel, Y., Seeman, P. and O'Dowd, B.F. (1990) Nature 347, 80–83.
[6] Gantz, I., Schaffer, M., DelValle, J., Logsdon, C., Campbell, V., Uhler, M. and Yamada, T. (1991) Proc. Natl. Acad. Sci. USA 88, 429–433.
[7] Rimland, J., Xin, W., Sweetnam, P., Saijoh, K., Nestler, E.J. and Duman, R.S. (1991) Mol. Pharmacol. 40, 869–875.
[8] Sugimoto, Y., Namba, T., Honda, A., Hayashi, Y., Negishi, M., Ichikawa, A. and Narumiya, S. (1992) J. Biol. Chem. 267, 6463–6466.
[9] Mountjoy, K.G., Robbins, L.S., Mortrud, M.T. and Cone, R.D. (1992) Science 257, 1248–1251.
[10] Tsutsumi, M., Zhou, W., Millar, R.P., Mellon, P.L., Roberts, J.L., Flanagan, C.A., Dong, K., Gillo, B. and Sealfon, S.C. (1992) Mol. Endocrinol. 6, 1163–1169.
[11] Murphy, T.J., Alexander, R.W., Griendling, K.K., Runge, M.S. and Bernstein, K.E. (1991) Nature 351, 233–236.
[12] Sargent, T.D., Wu, J.R., Sala-Trepat, J.M., Wallace, R.B., Reyes, A.A. and Bonner, J. (1979) Proc. Natl. Acad. Sci USA 76, 3256–3260.
[13] Harrison, J.K., D'Angelo, D.D., Zeng, D. and Lynch, K.R. (1991) Mol. Pharmacol. 40, 407–412.
[14] Durieux, M.E., Salafranca, M.N., Lynch, K.R. and Moorman, J.R. (1992) Am. J. Physiol. 263 (Cell Physiol. 32), C896–C900.
[15] Sambrook, J., Fritsh, F. and Maniatis, T. (1989) Molecular Cloning, Cold Spring Harbor Laboratory, New York.
[16] Church, G.M. and Gilbert, W. (1984) Proc. Natl. Acad. Sci. USA 81, 1991–1995.
[17] Lipman, D.J. and Pearson, W.R. (1985) Science 227, 1435–1441.
[18] McEachern, A.E., Shelton, E.R., Bhakta, S., Obernolte, R., Bach, C., Zuppan, P., Fujisaka, J., Aldrich, R.W. and Jarnagin, K. (1991) Proc. Natl. Acad. Sci. USA 88, 7724–7728.
[19] Kluxen, F.-W., Bruns, C. and Luebbert, H. (1992) Proc. Natl. Acad. Sci. USA 89, 4618–4622.
[20] Murphy, P.M. and Tiffany, H.L. (1991) Science 253, 1280–1283.
[21] Boulay, F., Tardif, M., Brouchan, L. and Vignais, P. (1990) Biochem. Biophys. Res. Commun. 168, 1103–1109.
[22] Gerard, N.P. and Gerard, C. (1991) Nature 349, 614–617.
[23] Devereux, J., Haeberli, P. and Smithies, O. (1984) Nucleic Acids Res. 12, 387–395.