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Cloning of the γ-aminobutyric acid (GABA) ρ_1 cDNA: A GABA receptor subunit highly expressed in the retina

(ligand-gated ion-channel receptors/polymerase chain reaction/chloride channels/gene family/oocyte expression)

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ABSTRACT **Type A γ-aminobutyric acid ($GABA_A$) receptors are a family of ligand-gated chloride channels that are the major inhibitory neurotransmitter receptors in the nervous system. Molecular cloning has revealed diversity in the subunits that compose this heterooligomeric receptor, but each previously elucidated subunit displays amino acid similarity in conserved structural elements. We have used these highly conserved regions to identify additional members of this family by using the polymerase chain reaction (PCR). One PCR product was used to isolate a full-length cDNA from a human retina cDNA library. The mature protein predicted from this cDNA sequence is 458 amino acids long and displays between 30 and 38% amino acid similarity to the previously identified $GABA_A$ subunits. This gene is expressed primarily in the retina but transcripts are also detected in the brain, lung, and thymus. Injection of *Xenopus* oocytes with RNA transcribed *in vitro* produces a GABA-responsive chloride conductance and expression of the cDNA in COS cells yields GABA-displaceable muscimol binding. These features are consistent with our identification of a GABA subunit, GABA ρ_1, with prominent retinal expression that increases the diversity and tissue specificity of this ligand-gated ion-channel receptor family.**

γ-Aminobutyric acid (GABA), the major brain inhibitory neurotransmitter, mediates fast synaptic inhibition by activating a chloride channel. The receptor belongs to a superfamily of ligand-gated ion channels that include the strychnine-sensitive glycine receptors, glutamate receptors, and nicotinic acetylcholine receptors (1–3). The type A GABA ($GABA_A$) receptor is postulated to be a heterooligomeric structure composed of various combinations of subunits belonging to at least four reported classes (α, β, γ, and δ) (3). Molecular cloning reveals further diversity within each class; six α, three β, and two γ subunits have been reported (1, 4–10). Most of the subunits can be activated by GABA when expressed individually in oocytes. Different subunit combinations can yield receptors displaying differing responses to a variety of ligands, particularly benzodiazepine and barbiturates (8, 11, 12). Considerable regional variation in the expression of each $GABA_A$ subunit in the brain is also observed (10, 13, 14).

Cloning of several $GABA_A$ subunits has demonstrated similarities in their predicted amino acid sequences that are highest in areas encoding four hydrophobic segments that are believed to form the chloride channel pore. Glycine receptors also form chloride channels and display homologies in these proposed channel-forming domains. These homologies are greater than those of ligand-gated channels that pass other ions, e.g., kainate or nicotinic acetylcholine (15, 16). We have, therefore, used the regions of greatest similarity among transmembrane segments 2 and 3 of the $GABA_A$ and glycine subunits to clone additional proteins encoding chloride channels. Subcloning and sequencing of products amplified from human genomic DNA and cDNA from a colonic tumor cell line (T84) believed to express the chloride channel defective in cystic fibrosis (17) have identified an additional GABA subunit, termed GABA ρ_1 that demonstrates unique pharmacological properties, and is primarily expressed in the retina.**

MATERIALS AND METHODS

Polymerase Chain Reaction (PCR) Amplification of Human DNA Sequences from the $GABA_A$/Glycine Receptor Gene Family. Primers for the PCR were selected from regions of high similarity among the amino acid sequences of the bovine α1 and β1 $GABA_A$ subunits (1) and the rat glycine receptor (2). Mixed oligonucleotides primer A [5′-AC(A,C)AC(A,T)-GTGCT(C,G)AC(A,C)ATCAC(A,C)AC-3′] was derived from the octomeric amino acid sequence TTVLTMTT in the second transmembrane region of the $GABA_A$ α1 and glycine subunits (1, 2). Mixed primer B [5′-CAG(A,G)GC(C,T)-GA(A,G)AA(C,T)AC(A,G)AA-3′] was selected from the conserved amino acid residues FVFSAL in the third transmembrane segment of the α1 $GABA_A$ and glycine receptor subunits. Genomic DNA for PCR amplification was derived from peripheral lymphocytes of healthy individuals or phage libraries (LL07NS01 and LA07NS01; American Type Culture Collection). cDNA was synthesized from 1 μg of mRNA isolated from a human colonic tumor cell line (T84) by using Moloney murine leukemia virus reverse transcriptase (BRL) and standard methods (18, 19).

The PCR was performed in 100 μl containing either 500–1000 ng of genomic DNA or 50–100 ng of cDNA, 2.5 units of *Taq* polymerase (Cetus), all four deoxynucleotide 5′-triphosphates (Pharmacia; each at 0.02 μM), and the 5′ and 3′ primers (each at 20 pM) in *Taq* polymerase buffer (50 mM KCl/10 mM Tris·HCl, pH 8.3/1.5 mM $MgCl_2$/0.01% gelatin). The DNA was amplified by denaturation at 94°C for 6 min, then by 30 cycles of annealing at 45°C for 1 min, extension at 72°C for 1 min, and denaturation at 94°C for 30 sec, and finally by an extension at 72°C for 10 min.

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Abbreviations: GABA, γ-aminobutyric acid; $GABA_A$ receptor, type A GABA receptor; PCR, polymerase chain reaction.

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**The sequence reported in this paper has been deposited in the GenBank data base (accession no. M62323).

Subcloning and Sequencing of PCR Amplification Products. To facilitate cloning, 123-base-pair (bp) fragments from each reaction mixture were reamplified using 100 pM of primers G and F, which are identical to primers A and B, respectively, but include a *Hin*dIII site at the 5′ end of primer G and a *Bam*HI site at the 5′ end of primer F. Amplified DNA fragments (140 bp) were subcloned into the pGEM-3Z+ plasmid (Promega) and recombinants were PCR-amplified using universal M13 sequencing primers. Direct sequencing of the PCR products using the Forward primer was performed as described (20).

Identification and Sequencing of the Full-Length cDNA. Seven cDNA libraries were screened for the cDNA corresponding to clone D by using standard methods (19). These included the following: a T84 library constructed in λgt10, a second T84 library (Clontech 1079k), a fetal human brain library, a 1-day-old human cortex library, an adult rat cortex library constructed in λZAP, an adult human lung library (Clontech 1066b), and a human retina library. Phage DNA from a single colony isolated from the human retina cDNA library was partially digested with *Eco*RI to yield a full-length cDNA, since an *Eco*RI site exists within the cDNA. The resultant 2.0-kilobase (kb) fragment was subcloned into the pBluescript KS+ vector (Stratagene) and designated pR5-2.0. Sequencing of the entire coding region (1.4 kb) of pR5-2.0 was performed in both directions by the dideoxynucleotide method using sequencing primers (21). Sequence translation and alignments were performed using the PC Gene V6.25 (IntelliGenetics) software package.

Expression of the Full-Length cDNA. Sense and antisense mRNA for injection into *Xenopus* oocytes was synthesized from the linearized pR5-2.0 plasmid using the T3 and T7 RNA polymerase promoters, respectively (19). Adult female *Xenopus laevis* frogs (Xenopus I, Ann Arbor, MI) were anesthetized in 0.15% tricaine methanesulfonate (Ayerst Laboratories), ovarian lobes were excised, oocytes were defolliculated, (22) and stage V and VI oocytes were selected and stored in modified Barth's solution [MBS; 88 mM NaCl/1 mM KCl/2.4 mM $NaHCO_3$/0.3 mM $Ca(NO_3)_2$/0.4 mM $CaCl_2$/0.8 mM $MgSO_4$/15 mM Tris·HCl, pH 7.6/penicillin (100 units/ml)/streptomycin (100 μg/ml)] at 18°C. Eighteen to 24 hours after defolliculation, oocytes were injected with 50 ng of mRNA in 50 nl of water with a positive-displacement micropipette (Drummond). Two-microelectrode voltage clamp experiments were performed in normal frog saline (96 mM NaCl/2 mM KCl/2 mM $CaCl_2$/1 mM $MgCl_2$/5 mM Hepes·NaOH, pH 7.4) (22).

To facilitate expression in mammalian cells, the 2.0-kb fragment from pR5-2.0 was subcloned into *Not* I–*Xho* I sites in pCDM8 (Invitrogen, San Diego). This construct was transfected into COS cells by using electroporation (23). GABA-displaceable binding of [^{3}H]muscimol to COS cell membrane homogenates was determined by a filtration assay in cells harvested 3 days after transfection (24).

RESULTS

Identification of Sequences from the M2–M3 Regions of Human $GABA_A$ Receptor Genes. Analysis of 101 clones derived from PCR amplification of genomic DNA and human colonic tumor cell (T84) cDNA using primers A and B produced 12 sequences. Seven of the 12 sequences (clones A to F and H) showed various degrees of amino acid similarity with the M2–M3 regions of the $GABA_A$ and glycine subunits when translated in the same frame as the $GABA_A$/glycine receptor genes (Fig. 1*A*). All 7 sequences were amplified from human genomic DNA whereas only clones A, C, and D were amplified from T84 cDNA. Analysis of the relatedness of the 7 clones, the $GABA_A$ receptor subunits, and the 48-kDa α1 glycine receptor subunit (Fig. 1*B*) indicates that clones A, E, F, and C belong to the $GABA_A$ α subunit class. The sequence of clone E is identical to that of the human α1 $GABA_A$ subunit (25); it is possible that clone A is derived from the human α2 subunit gene. Clone F is also closely related to the α1/α2 subunits and may represent another member of this group. Clone C may be the human α6 subunit because it has only a single amino acid difference from the bovine α6 subunit and 87% similarity at the nucleotide level. Clones H and B correspond to human $GABA_A$ β subunits, clone H is identical to the human β1 subunit (25), and clone B most closely resembles the rat β3 subunit.

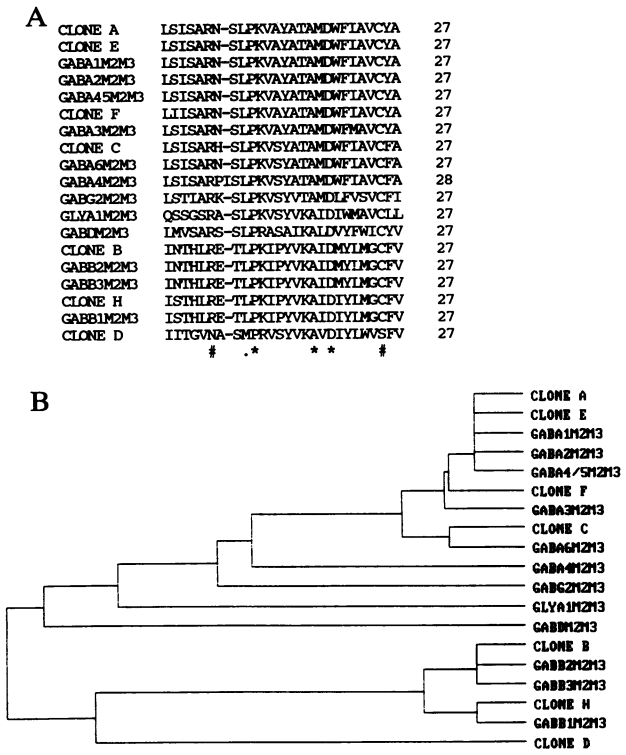

FIG. 1. Alignment of the predicted amino acid sequence of PCR-amplified sequences clones A to F and H with comparable regions (transmembrane segments M2–M3) from the $GABA_A$ α_1 (GABA1M2M3; ref. 25), α_2 (GABA2M2M3; ref. 4), α_3 (GABA3M2M3; ref. 4), α_4 (GABA4M2M3; ref. 5), α_4/α_5 (GABA45M2M3; refs. 6 and 7), α_6 (GABA6M2M3; ref. 8), $\beta 1$ (GABB1M2M3; ref. 25), β_2 (GABB2M2M3; ref. 9), β_3 (GABB3M2M3; ref. 9), γ_2 (GABG2M2M3; ref. 11), δ (GABDM2M3; ref. 10), and glycine α_1 (GLYA1M2M3; ref. 26) subunits using the program Clustal (PC Gene; IntelliGenetics). Residues that are completely conserved are indicated (*). Residues that are the same in all sequences except clone D are identified for a conservative change (·) and for nonconservative changes (#). (*B*) Phylogenetic tree generated during alignment of the amino acid sequences in *A*. Sequences on the same vertical line are identical, and sequences separated by a branch have at least one amino acid difference.

Clone D is distinct. The clone is similar to the $GABA_A$ and glycine receptors and is most closely related to the $GABA_A$ β subunits. However, it displays the lowest amino acid similarity with the known subunits. Specifically, three residues that are completely conserved among the $GABA_A$ and the glycine receptor subunits are different in clone D (Fig. 1*A*). These features all suggest that clone D is related to the $GABA_A$ and glycine receptors but represents another subclass of these proteins (Fig. 1*B*).

Cloning and Sequencing of the GABA ρ_1 Subunit. Screening of 3×10^6 phage from two T84 cDNA libraries, 1×10^6 phage from a human adult lung cDNA library, 1×10^6 phage from a human fetal brain cDNA library, 0.3×10^6 primary recombinants from a 1-day old-human cortex cDNA library, and 2

$\times 10^6$ phage from an adult rat cortex library with clone D failed to identify a cDNA clone containing the clone D sequence. However, screening of a human retina cDNA library (1×10^6 recombinants) identified eight positively hybridizing cDNA clones. Partial sequencing demonstrated that all eight clones contained sequence from the same gene. The complete sequence of the longest insert (2.0 kb) has an open reading frame of 1422 bp encoding a predicted protein of 473 amino acids (Fig. 2). Translation is predicted to initiate at the second, in-frame AUG codon because the first AUG (nucleotide 29) lacks a highly conserved purine at position −3 of the eukaryotic ribosome binding consensus sequence (30). An in-frame nonsense signal is present in the proposed 5′ untranslated region (nucleotides 13–15), indicating that translation does not initiate prior to this region. A 15-amino acid signal peptide sequence is predicted to be cleaved to produce a mature protein of 458 residues. Alignment of the proposed amino acid sequence of this cDNA with representative subunits from the four $GABA_A$ classes is shown in Fig. 3. The high degree of similarity, especially within the transmembrane regions, is consistent with membership of this cDNA

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                                        +++
                                cgagaaggatgtttgaatttggaaacccatgttggctgtcccaaat

 47  ATG AGA TTT GGC ATC TTT CTT TTG TGG TGG GGA TGG GTT TTG GCC ACT GAA AGC
  1  MET Arg Phe Gly Ile Phe Leu Leu Trp Trp Gly Trp Val Leu Ala Thr Glu Ser
                                                  ↑
101  AGA ATG CAC TGG CCC GGA AGA GAA GTC CAC GAG ATG TCT AAG AAA GGC AGG CCC
 19  Arg Met His Trp Pro Gly Arg Glu Val His Glu Met Ser Lys Lys Gly Arg Pro
                                                 *
155  CAA AGA CAA AGA CGA GAA GTA CAT GAA GAT GCC CAC AAG CAA GTC AGC CCA ATT
 37  Gln Arg Gln Arg Arg Glu Val His Glu Asp Ala His Lys Gln Val Ser Pro Ile

209  CTG AGA CGA AGT CCT GAC ATC ACC AAA TCG CCT CTG ACA AAG TCA GAA CAG CTT
 55  Leu Arg Arg Ser Pro Asp Ile Thr Lys Ser Pro Leu Thr Lys Ser Glu Gln Leu

263  CTG AGG ATA GAT GAC CAT GAT TTC AGC ATG AGG CCT GGC TTT GGA GGC CCT GCC
 73  Leu Arg Ile Asp Asp His Asp Phe Ser Met Arg Pro Gly Phe Gly Gly Pro Ala
                                      *
317  ATT CCT GTT GGT GTG GAT GTG CAG GTG GAG AGT TTG GAT AGC ATC TCA GAG GTT
 91  Ile Pro Val Gly Val Asp Val Gln Val Glu Ser Leu Asp Ser Ile Ser Glu Val

371  GAC ATG GAC TTT ACG ATG ACC CTC TAC CTG AGG CAC TAC TGG AAG GAC GAG AGG
109  Asp Met Asp Phe Thr Met Thr Leu Tyr Leu Arg His Tyr Trp Lys Asp Glu Arg

425  CTG TCT TTT CCA AGC ACC AAC AAC CTC AGC ATG ACG TTT GAT GGC CGG CTG GTC
127  Leu Ser Phe Pro Ser Thr Asn Asn Leu Ser Met Thr Phe Asp Gly Arg Leu Val
                                 ^^^^^^^^^^
                                 ^^^^^^^^^^
479  AAG AAG ATC TGG GTC CCT GAC ATG TTT TTC GTG CAC TCC AAA CGC TCC TTC ATC
145  Lys Lys Ile Trp Val Pro Asp Met Phe Phe Val His Ser Lys Arg Ser Phe Ile
                                            *
533  CAC GAC ACC ACC ACA GAC AAC GTC ATG TTG CGG GTC CAG CCT GAT GGG AAA GTG
163  His Asp Thr Thr Thr Asp Asn Val Met Leu Arg Val Gln Pro Asp Gly Lys Val

587  CTC TAT AGT CTC AGG GTT ACA GTA ACT GCA ATG TGC AAC ATG GAC TTC AGC CGA
181  Leu Tyr Ser Leu Arg Val Thr Val Thr Ala Met Cys Asn Met Asp Phe Ser Arg
          *                                  |-------------------
641  TTT CCC TTG GAC ACA CAA ACG TGC TCT CTT GAA ATT GAA AGC TAT GCC TAT ACA
199  Phe Pro Leu Asp Thr Gln Thr Cys Ser Leu Glu Ile Glu Ser Tyr Ala Tyr Thr
     ------------------------|
695  GAA GAT GAC CTC ATG CTG TAC TGG AAA AAG GGC AAT GAC TCC TTA AAG ACA GAT
217  Glu Asp Asp Leu Met Leu Tyr Trp Lys Lys Gly Asn Asp Ser Leu Lys Thr Asp
                                    ^^^^^^^^^*^
749  GAA CGG ATC TCA CTC TCC CAG TTC CTC ATT CAG GAA TTC CAC ACC ACC ACC AAA
235  Glu Arg Ile Ser Leu Ser Gln Phe Leu Ile Gln Glu Phe His Thr Thr Thr Lys
                                                  *
803  CTG GCT TTC TAC AGC AGC ACA GGC TGG TAC AAC CGT CTC TAC ATT AAT TTC ACG
253  Leu Ala Phe Tyr Ser Ser Thr Gly Trp Tyr Asn Arg Leu Tyr Ile Asn Phe Thr
                                                  ^^^^^^^^^*^
857  TTG CGT CGC CAC ATC TTC TTC TTC TTG CTC CAA ACT TAT TTC CCC GCT ACC CTG
271  Leu Arg Arg His Ile Phe Phe Phe Leu Leu Gln Thr Tyr Phe Pro Ala Thr Leu

911  ATG GTC ATG CTG TCC TGG GTG TCC TTC TGG ATC GAC CGC AGA GCC GTG CCT GCC
289  Met Val Met Leu Ser Trp Val Ser Phe Trp Ile Asp Arg Arg Ala Val Pro Ala

965  AGA GTC CCC TTA GGT ATC ACA ACG GTG CTG ACC ATG TCC ACC ATC ATC ACG GGC
307  Arg Val Pro Leu Gly Ile Thr Thr Val Leu Thr Met Ser Thr Ile Ile Thr Gly

1019 GTG AAT GCC TCC ATG CCG CGC GTC TCC TAC ATC AAG GCC GTG GAC ATC TAC CTC
325  Val Asn Ala Ser Met Pro Arg Val Ser Tyr Ile Lys Ala Val Asp Ile Tyr Leu
                ^^^^^^^^^^
1073 TGG GTC AGC TTT GTG TTC GTG TTC CTC TCG GTG CTG GAG TAT GCG GCC GTC AAC
343  Trp Val Ser Phe Val Phe Val Phe Leu Ser Val Leu Glu Tyr Ala Ala Val Asn

1127 TAC CTG ACC ACT GTG CAG GAG AGG AAG GAA CAG AAG CTG CGG GAG AAG CTT CCC
361  Tyr Leu Thr Thr Val Gln Glu Arg Lys Glu Gln Lys Leu Arg Glu Lys Leu Pro

1181 TGC ACC AGC GGA TTA CCT CCG CCC CGC ACT GCA ATG CTG GAC GGC AAC TAC AGT
379  Cys Thr Ser Gly Leu Pro Pro Pro Arg Thr Ala Met Leu Asp Gly Asn Tyr Ser
                                                  ^^^^^^^^^^
1235 GAT GGG GAG GTG AAT GAC CTG GAC AAC TAC ATG CCA GAG AAT GGA GAG AAG CCC
397  Asp Gly Glu Val Asn Asp Leu Asp Asn Tyr Met Pro Glu Asn Gly Glu Lys Pro

1289 GAC AGG ATG ATG GTG CAG CTG ACC CTG GCC TCA GAG AGG AGC TCC CCA CAG AGG
415  Asp Arg Met Met Val Gln Leu Thr Leu Ala Ser Glu Arg Ser Ser Pro Gln Arg
                                      *
1343 AAA AGT CAG AGA AGC AGC TAT GTG AGC ATG AGA ATC GAC ACC CAC GCC ATT GAT
433  Lys Ser Gln Arg Ser Ser Tyr Val Ser Met Arg Ile Asp Thr His Ala Ile Asp
      *                           *
1397 AAA TAC TCC AGG ATC ATC TTT CCA GCA GCA TAC ATT TTA TTC AAT TTA ATA TAC
451  Lys Tyr Ser Arg Ile Ile Phe Pro Ala Ala Tyr Ile Leu Phe Asn Leu Ile Tyr

1451 TGG TCT ATT TTC TCC TAG atgcttgtaattctacaaatttcacatttccatggcatgcactacag
469  Trp Ser Ile Phe Ser ---

1516 aaataactgtataatgaaaaagtatttaaggatatggttaaaaaaaaatcccaggacccacccatgttttc
1587 actatcccttctgcagctttccaaagctacattgacgagacacttactggtttaatttgcacttattaacc
1658 tctgttgaatacacagcattatattaggtgctgcagaaatacgacactgtagcgactgatgttagttgtta
1730 cccagataaaatggaaaagcacactaccagtgttgtgggcacatttagxtccacccgattagacccttgat
1801 gctattcacatgaataatttatttttccctaaaagtgtcattacattgttcaggctacgtgaacttggaag
1872 caccatcaggccatttgcatgaaattcacatgcacctaaatcctcactttgacagaaactcatgcttcagt
1943 tataacctattacctattttgtatgcgactccacctccgcatgttcg
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FIG. 2. Nucleotide and predicted amino acid sequence of the GABA ρ_1 cDNA. Amino acids are shown in three-letter code below the nucleotide sequence. The in-frame stop codon within the 5′ untranslated region is indicated by (+++). The predicted site of cleavage to form a mature protein is shown by (↑) (27), potential protein kinase C phosphorylation sites (28) are indicated by (*), putative asparagine glycosylation sites are designated (^^^^^^) (29), the Cys–Cys loop is indicated by a dashed line, and proposed transmembrane regions are indicated by underlining. A single undetermined base in the 3′ untranslated region is indicated by an x.

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GABA1$HUM                                          QPSLQDELKDNTTVFTRI   18
GABG2$HUM                                  QKSDDDYEDYASWKIWVLITPKVPEGDVIVI   30
GABB1$HUM                                          HSTNEPSNMPYVKETVDR   18
GABD$RAT                                   QPHHGARAMNDIGDYVGSNLEISWLPNLDG   30
GABR1$HUM  TESRMHWPGREVHEMSKKGRPQRQRREVHEDAHKQVSPILRRSPDITKSPLITKSE   55

                 #  ##    **  *          * *        * .*        * *
GABA1$HUM  LDRLLDGYDNRLRPGLGERVTEVKTDIFVTSFGPVSDHDMEYTIDVFFRQSWKDE   73
GABG2$HUM  LNNLLEGYDNKLRPDIGVKPTLIHTDMVVNSIGPVNAINMEYTIDIFFAQWWDR   85
GABB1$HUM  ---LLKGYDIRLRPDFGGPPVDVGMRIDVASIDMVSEVNMDYTLTMYFQQSWKDK   70
GABD$RAT   ---IMEGYARNFRPGIGGPPVNVALALEVASIDHISEANMEYTMIVFLHRAWRDS   82
GABR1$HUM  QLLRIDDHDFSMRPGFGGPAIPVGVDVQVESLDSISEVDMDFTMTLYLRHYWKDE   110

           **         . #          **#   # *    *  *  *   *   **
GABA1$HUM  RLKFKGPMIV-LRLNNLMASKIRIPDIFFPHNGKKSVAHNMIMPNKLLRITEDGTL   127
GABG2$HUM  RLKFNSTIKV-LRLNSNMVGKIWIPDTFFRNSKKADAHWITTPNRMLRIWNDGRV   139
GABB1$HUM  RLSYSGIP-LNLITLDNRVADQLWVPDTYFLNDKKSFVHGVTVKNRMIRLHPDGTV   124
GABD$RAT   RLSYNHIN-ETLGLDSRFVDKLWLPDTFIVNAKVCLVHDVTVENKLIRLQPDGVI   136
GABR1$HUM  RLSFPSTNNLSMTFDGRLVKKIWVPDMFFVHSKRSFIHDITTIDNVMLRVQPDGKV   165

           ** * *    *   #   * *   * *   ** *        *
GABA1$HUM  LYTMRLTVRAECPMHLEDFPMDAHACPLKFGSYAYTRAEVVYEWTREPARSVVVA   182
GABG2$HUM  LYSLRLTIDAECQLQLHNFPMDEHSCPLEFSSYGYPREEIVYQWKRSSVE---VGD   192
GABB1$HUM  LYGLRITTTAACMMDLRRVPLDEQNCTLEIESYGYTTDDIEFYWNGG---EGAVTG   177
GABD$RAT   LYSIRITTSTVACDMDLAKVPMDEQECMLDLESYGYSSEDIVYYWSEN---QEQIHG   189
GABR1$HUM  LYSLRVTVIAMCNMDFSRFPLDTQTCSLEIESYAYTEDDIMLYWKKG---NDSLKT   218
                     |-------------|

             * *                 *        * * *  #    * * *
GABA1$HUM  EDGSRLNQYDLLGQTVDSGIVQ-SSTGEYVVMTTHFHLKRKIGYFVIQTYLPCIM   236
GABG2$HUM  TRSWRLYQFSFVGLRNTTEVVK-TTSGDYVVMSVYFDLSRRMGYFTIQTYIPCTL   246
GABB1$HUM  VNKIELPQFSIVDYKMVSKKVEF-TTGAYPRLSLSFRLKRNIGYFILQTYMPSTL   231
GABD$RAT   LDRLQLAQFTTTSYRFTTELMNFKSAGQFPRISLHFQLRRNRGVVIIQSVMPSVL   244
GABR1$HUM  DERISLSQFLIQEFHTITTKLAFYSSTGWYNRLYINFTLRRHIFFFLLQTYFPATL   273
                                                    ═══════════

             * ****        **   * ******.*     #  .*       * *
GABA1$HUM  TVILSQVSFWLNRESVPARTVPGVTTVLTMTTLSISARNSLPKVAYATAMDWFIA   291
GABG2$HUM  IVVLSWVSFWINKDAVPARTSLGITTVLTMTTLSTIARKSLPKVSYVTAMDLFVS   301
GABB1$HUM  ITILSWVSFWINYDASAARVALGITTVLTMTTISTHLRETLPKIPYVKAIDIYLM   286
GABD$RAT   LVAMSWVSFWISQAAVPARVSLGITTVLTMTTLMVSARSSLPRASAIKALDVYFW   299
GABR1$HUM  MVMLSWVSFWIDRRAVPARVPLGITTVLTMSTIITGVNASMPRVSYIKAVDIYLW   328
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             #  *** .. *
GABA1$HUM  VCYAFVFSALIEFATVNYFTKRGYAWDGKSVVPEKPKKVKDPLIKKNNTYAPTAT   346
GABG2$HUM  VCFIFVFSALVEYGTLHYFVSNRKPSKDKDKKKKNPAPTIDIRPRSATIQMNNAT   356
GABB1$HUM  GCFVFVFLALLEYAFVNYIFFGKGPQKKGASKQDQSANEKNKLEMNKVQVDAHGN   341
GABD$RAT   ICYVFVFAALVEYAFAHF----------NADYRKKRKAVKVTKPRAGMDVRNA   343
GABR1$HUM  VSFVFVFLSVLEYAAVNYL----------TTVQERKEQKLREKLPCTSGLPPPRTA   374
           ═══ ═══════════                                ═══

GABA1$HUM  SYTPNLARGD----------PGLATIAK   364
GABG2$HUM  HLQERDEEYG----------YECLDGKD   374
GABB1$HUM  ILLSTLEIRNETSGSEVLITSVSDPKAIMYSYDSASIQYRKPLSSREAYGRAIDRH   396
GABD$RAT   IVLFSLSAAGVSQELAISRRQGRVPGNLMGSYRSVEVEAKKEGGVPPGGPGGIRS   398
GABR1$HUM  MLDGNYSDGEVNDLDNYMPENGEKPDRMM----------VQL   406

                                  *  *  **  .  *  **  .
GABA1$HUM  SATIEPKEVKPETKPPEPKKTFNSVSKIDRLSRIAFPLLFGIFNLVYWATYLNRE   419
GABG2$HUM  CASFFCCFEDCRTGAWRHGRIHIRIAKMDSYARIFFPTAFCLFNLVYWVSYLYL   428
GABB1$HUM  GVPSKGRIRRRASQLKVKIPDLITDVNSIDKWSRMFFPITFSLFNVVYWLYYVH   449
GABD$RAT   RLKP----------IDADTIDIYARAVFPAAFAAVNIIYWAAYTM   433
GABR1$HUM  TLASERSSPQRKSQRSSYVSMRIDTHAIDKYSRIIFPAAYILFNLIYWSIFS   458
                                                    ═══════════

GABA1$HUM  PQLKAPTPHQ   429
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FIG. 3. Alignment of the mature human $GABA_A$ subunits α_1 (GABA1$HUM) (25), $\beta 1$ (GABB1$HUM) (25), and $\gamma 2$ (GABG2$HUM) (11) and the rat δ subunit GABD$RAT (10) with the human GABA ρ_1 (GABR1$HUM). Residues that are completely conserved are indicated (*). Residues that are the same in all sequences except GABA ρ_1 are identified for conservative changes (·) and for nonconservative changes (#). The Cys–Cys loop is shown by a dashed line and proposed transmembrane regions are shown with double underlining.

in the GABA receptor family and has been designated as subunit ρ_1.

Tissue Distribution of the GABA ρ_1 Subunit. Northern blot analyses of poly(A)-selected RNA extracted from several brain regions and peripheral tissues demonstrated hybridization to an mRNA of 3.9 kb in bovine retina and cerebellum. Bovine retina also contained at least two additional hybridizing bands at 3.1 and 4.8 kb (Fig. 4). Much lower or undetectable levels of hybridization were found in RNA prepared from rat and guinea pig cerebellum, guinea pig and human fetal forebrain, bovine and rat midbrain, human fetal and bovine hindbrain, human adult caudate/putamen, rat and bovine cerebral cortex, bovine spinal cord, rat lung, and rat thymus (Fig. 4 and data not shown). Three weakly hybridizing transcripts were detected in 68 μg of mRNA from T84 cells (data not shown).

***Xenopus* and COS Cell Expression of GABA ρ_1.** Superfusion of 1 μM GABA onto oocytes injected with sense RNA transcribed from the GABA ρ_1 cDNA induced an inward current (78 recordings from 15 oocytes) (Fig. 5 *A–C*). The current was eliminated by removing GABA from the bath solution and as much as 2 mM glycine had no effect (n = 3). In the presence of 1 μm GABA, addition of 200 nM picrotoxin inhibited the current by as much as 80% (n = 5). Concentrations of picrotoxin of 0.5 to 10 μM completely inhibited GABA-activated currents (n = 10). No GABA-activated currents were observed in noninjected (n = 5) oocytes or in those injected with antisense RNA (n = 3). Current–voltage relationship of the GABA-activated current revealed a measured reversal potential of −35 mV with 120 mM chloride in the extracellular solution (Fig. 5*D*). The calculated reversal potential was −32 mV (33). The reversal potential shifted to −18 mV when extracellular Cl^- was reduced to 50 mM (n = 3). Currents were intermediate at 0.5 μM GABA and saturated at 10 μM GABA. The calculated concentration for half-maximal activation was 2 μM.

Cell membranes prepared from cells transfected with the GABA ρ_1 cDNA demonstrated GABA-displaceable muscimol binding. The binding affinity of this homooligomeric receptor was low [K_d = 0.4 to 1.0 μM (n = 3)]. Displacement of muscimol binding by GABA was not observed in cell membranes isolated from pCDM8-transfected or nontransfected cells.

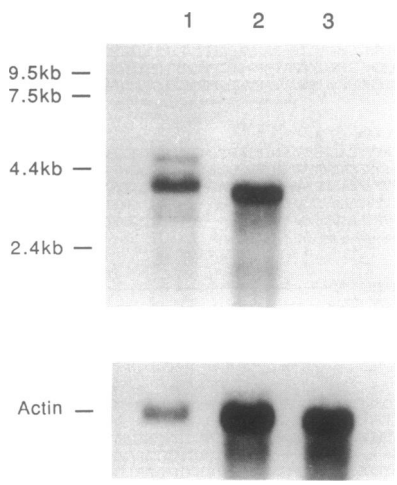

FIG. 4. Northern blot analysis for the presence of GABA ρ_1 mRNA. Lanes: 1, bovine retina [1 μg of poly(A)$^+$ RNA]; 2, bovine cerebellum [10 μg of poly(A)$^+$ RNA]; 3, bovine cerebral cortex [10 μg of poly(A)$^+$ RNA] hybridized at 37°C for 16–24 hr with a 400-bp *Eco*RI–*Hin*dIII cDNA fragment (corresponding to transmembrane regions 1–3) radiolabeled by random priming (31) in a standard 50% formamide buffer (19). Below, the same blot probed with a β-actin cDNA (32). Filters were washed for three 20-min periods in 0.4 × standard saline/citrate/0.5% SDS at 48°C and autoradiographed at −70°C for 1–6 days using an intensifying screen. An RNA ladder (BRL) was used to standardize RNA sizes.

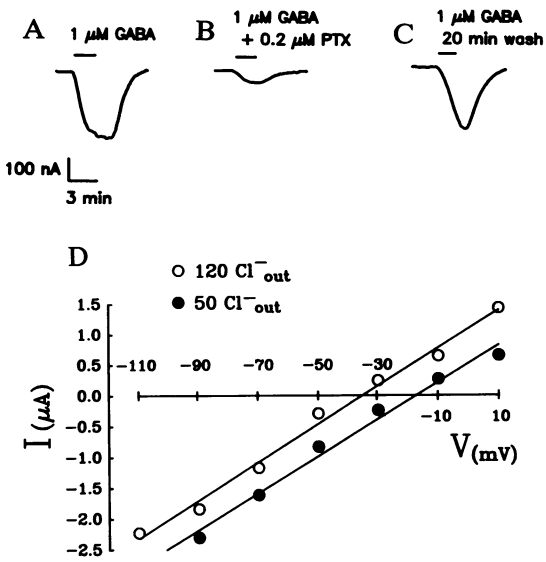

FIG. 5. Expression of the GABA ρ_1 receptor in *Xenopus* oocytes injected with sense mRNA derived from clone pR5-2.0. GABA-activated membrane currents were evoked by perfusion of 1 μM GABA (*A*); 1 μM GABA plus 200 nM picrotoxin (PTX) (*B*); or 1 μM GABA, after washing out the picrotoxin for 20 min (*C*). Membrane potentials were clamped at −90 mV, and bars show the perfusion period of GABA in the extracellular solution. (*D*) Current (*I*)–voltage (*V*) relationship of the GABA-activated currents (10 μM GABA applied in extracellular solution).

DISCUSSION

Amplification of human $GABA_A$ genes by using PCR and mixed oligonucleotides for conserved regions has identified additional members of the GABA receptor gene family. In addition to isolation of sequences from potential members of the α and β subunit groups, an entire cDNA has been cloned and expressed that represents another class of GABA receptor subunits. We have also identified human counterparts for portions of some bovine and rat $GABA_A$ receptor genes. This method appears to be useful for any closely related family of genes, as demonstrated by the amplification of guanine nucleotide binding (G) proteins, P-glycoproteins, ATP kinase-dependent transport proteins, and the rat δ $GABA_A$ subunit by similar procedures (34–38).

Several subunits of $GABA_A$ receptors have been expressed in *Xenopus* oocytes (1, 10) and have shown ligand-gated chloride channel activity. Injection of ρ_1 mRNA into *Xenopus* oocytes demonstrates that the protein can form a homooligomeric receptor responsive to GABA. Transfection of mammalian cells with the ρ_1 cDNA confers GABA-displaceable muscimol binding to the cell membranes, confirming that the product of this cDNA can interact with GABA. In *Xenopus* oocytes, the cloned subunit displays dose–response relationships to GABA agonists similar to those displayed by cloned bovine α and β subunits (1) and a much lower half-maximal GABA concentration than chicken brain $GABA_A$ receptors (39). Compared with other GABA-activated chloride channels expressed singly in oocytes, this cloned subunit has a higher sensitivity to picrotoxin, a convulsant inhibitor of GABA-activated chloride channels. A picrotoxin concentration of 0.5 to 10 μM completely inhibits responses after ρ_1 subunit mRNA injection whereas 5–500 μM is required for $GABA_A$ receptors (4, 26, 40). This indicates the ρ_1 subunit has a distinct pharmacologic character. Chloride ion substitution

experiments (Fig. 5*D*) confirm that that ρ_1 subunit contains a chloride-selective anion channel.

Each of the four cloned $GABA_A$ subunits demonstrates distinct regional variations of expression within the brain (3). $GABA_A$-responsive chloride channels have been detected in the pancreas and retina, indicating that they are also present in tissues outside of the central nervous system (41, 42). Our experimental results suggest that expression of the GABA ρ_1 gene in retina is substantially higher than expression in brain regions. Although four brain libraries were screened using clone D, no hybridizing cDNA were obtained. Conversely, a human retina cDNA library yielded a 2.0-kb cDNA in the first screen, among eight positive λgt10 plaques. These results were consistent with the results of a Northern blot analysis of RNA prepared from the retina and other brain regions. The hybridization seen after Northern blot analysis of as little as 1 μg of retinal poly(A) RNA was significantly higher than the expression in the next-highest brain region tested, the bovine cerebellum, and dramatically higher than other regions tested. This relatively selective distribution leads us to propose that this receptor cDNA can be designated the ρ subunit class to denote its relative, although not absolute, retina-specific localization.

Clone D corresponding to the GABA ρ_1 gene was amplified from cDNA created from the T84 cell line raising the possibility that GABA ρ_1 might be present in colonic cells. However, hybridization of T84 mRNA with a GABA ρ_1 probe revealed only faint signals, indicating very low levels of transcription of this gene in T84 cells. Clones A and C, probably $GABA_A$ α subunits, and β globin sequences (G.R.C., unpublished observations) have also been amplified from T84 cDNA. This suggested that a number of tissue-specific genes are transcribed at levels detectable by PCR amplification in T84 cells. This phenomenon has been observed for other cell types (43, 44). The low intensity of hybridization of RNA from lung and thymus with the GABA ρ_1 cDNA may reflect low abundance of this mRNA in these tissues or that only a small fraction of cells from these tissues express the ρ_1 gene.

In the retina, GABA is contained within retinal amacrine cells where it has been linked to inhibition processes and may even play a role in "straight-through" transmission of information on the path to retinal ganglion cells (45). Ligand-binding and physiological studies suggest that both $GABA_A$ and $GABA_B$ receptors are present in the retina (45). It is thus possible that the ρ_1 GABA receptor subunit could play a role in retinal neurotransmission. Preliminary *in situ* hybridization analysis of rat retina is consistent with the presence of this receptor subunit mRNA in retinal cell subpopulations (S.S. and G.R.U., unpublished data). The availability of the ρ_1 GABA receptor subunit cDNA will help elucidate the roles that these receptors may play in retinal information processing.

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