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Journal of Neurochemistry
Lippincott–Raven Publishers, Philadelphia

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Abstract: GABA-gated chloride channels are the main inhibitory neurotransmitter receptors in the CNS. Conserved domains among members of previously described $GABA_A$ receptor subunits were used to design degenerate sense and antisense oligonucleotides. A PCR product from this amplification was used to isolate a full-length cDNA. The predicted protein has many of the features shared by other members of the ligand-gated ion channel family. This channel subunit has significant amino acid identity (25–40%) with members of $GABA_A$ and $GABA_C$ receptor subunits and thus may represent a new subfamily of the GABA receptor channel. Although we cannot rule out that this clone encodes a receptor for an unidentified ligand, it was termed GABA χ. This gene is mainly expressed in placenta and in heart; however, placenta appears to express only an unspliced mRNA. In situ hybridization reveals that the GABA χ subunit mRNA is present in the electrical conduction system of the human heart. Our results suggest that novel GABA receptors expressed outside of the CNS may regulate cardiac function. **Key Words:** GABA receptors—Conduction system—Heart—Reverse transcriptase—PCR—In situ hybridization.
J. Neurochem. **68,** 1382–1389 (1997).

The ligand-gated ion channels that are activated by GABA, acetylcholine, glycine, and serotonin share several features (Noda et al., 1982; Grenningloh et al., 1987; Schofield et al., 1987; Maricq et al., 1991): A large NH_2-terminal extracellular domain containing a signature consensus sequence, the Cys–Cys loop, four hydrophobic transmembrane domains (M1–M4), and a long cytoplasmic loop connecting M3 and M4. The ligand-gated channels are pentameric membrane proteins comprised of homologous subunits (Langosch et al., 1988; Cooper et al., 1991). By analogy, it is assumed that the GABA-gated chloride channels are also pentameric (Nayeem et al., 1994). Single subunits from some members of this family can form functional homomeric receptors, but they typically lack properties of the native multisubunit receptor (Schofield et al., 1987).

GABA is the major inhibitory neurotransmitter in the CNS. However, GABA is also present in peripheral tissues where it acts on GABA receptors identical to those found in the CNS (Rorsman et al., 1988; Boué-Grabot et al., 1995). Different subtypes of GABA-gated chloride channels are generated by coassembly of subunits $\alpha 1-6$, $\beta 1-3$, $\gamma 1-3$, δ ($GABA_A$), and $\rho 1-3$ ($GABA_C$) (for review, see Sieghart, 1995). Subunits are grouped according to the degree of sequence identity. For example, the six α subunits are ~70% identical, but only 25–30% identical to β subunits (DeLorey and Olsen, 1992). Glycine receptors also form chloride channels, and their subunits are similar to those of the GABA receptors (Grenningloh et al., 1987). These similarities are mainly found in the transmembrane domains believed to form the Cl^- channel. We set out to clone new chloride channels; consensus sequences for the transmembrane domains of GABA/glycine receptor subunits were used to design degenerate oligonucleotides, which were then used as primers in the PCR.

We report here the cDNA sequence of an mRNA, expressed in human heart, encoding a protein that contains the hallmarks of ligand-gated channel subunits. The predicted protein displays strong similarities to $GABA_A$ and $GABA_C$ receptor subunits and was termed GABA χ. It is highly expressed in the electrical conduction system of the heart.

Received September 2, 1996; revised manuscript received December 9, 1996; accepted December 9, 1996.

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Abbreviations used: ORF, open reading frame; RT, reverse transcription.

MATERIALS AND METHODS

PCR amplification

Primers for the PCR were selected from the second (M2) and third (M3) transmembrane domains, which are highly conserved among the $GABA_A$ and glycine receptor subunits.

Mixed oligonucleotide primers M2: 5′ TCGAATTC ACNATGACNAC were derived from amino acid sequence TMTT. Mixed primers M3: 5′ CAAAGCTT (A,G) AA (G,T,C) AC (A,G) AA were derived from amino acid sequence FVF. In the oligonucleotide sequences, N stands for G+A+T+C and underlined nucleotides indicate 5′-extensions that include the *Eco*RI restriction enzyme site for M2 and the *Hin*dIII site for M3. PCR amplification was performed in a final volume of 50 μl containing 1 U of Taq polymerase (Cetus) in the buffer supplied by the manufacturer, with 1 μl cDNA obtained from T84 RNA, 1 μ*M* concentration of each primer, and 0.2 m*M* concentration of each deoxyribonucleotide. Amplification was performed according to the following schedule: first, three cycles of denaturation 60 s at 95°C, annealing 30 s at 29°C, and an extension for 30 s at 72°C, followed by 30 cycles of 30 s at 95°C, 30 s at 50°C, and 30 s at 72°C. The amplified products were then separated on a 6% acrylamide gel. A gel slice containing the band size ranging from 90 to 130 bp was cut out and incubated in 20 μl of water; 5 μl of the eluate was used for PCR amplification as described above, except that the first three cycles were omitted. The amplified cDNA was ethanol-precipitated, digested with *Eco*RI and *Hin*dIII restriction enzymes, and ligated into pBluescript (Stratagene). The recombinant DNA was transfected into *E. coli* and individual clones were analyzed by enzymatic sequencing (Sanger et al., 1977).

PCR analysis of RNA expression

Total RNA from the sinus node of a 20-week-old human fetus was isolated by the method of Chomczynski and Sacchi (1987). cDNAs were synthesized in 20 μl of reverse transcription mixture using superscript reverse transcriptase (GibcoBRL). Subsequently, 1 μl of sinus node cDNA was amplified by PCR; 1 μl of a λgt10 human fetal heart library (Clontech) was likewise amplified. The primers used for the GABA χ PCR were (5′-3′) C3 (reverse): AGATGATGTCAATGGTGTATTCCATG and C4 (forward): TCCCGTGATGTTGTCTATGGC. PCR products were generated by 1 U of Taq DNA Polymerase (Appligene, Illkirch, France) in the manufacturer's buffer. Reactions were performed for 35 cycles: 94°C, 30 s; 60°C, 30 s; and 72°C, 30 s in a triothermoblock apparatus (BIOMETRA, Göttingen, Germany). The primers used for the human tryptophanyl-tRNA (Frolova et al., 1991) synthetase were HWRS1: GTGAAAGAGTTCATGACTC, HWRS2: ACCTACTGGCTGACAGGAG, and HWRS3: AGGGCAGAGTGCTCCAGAGGA. Reactions were performed for 35 cycles: 94°C, 30 s; 56°C, 30 s; and 72°C, 90 s with primers HWRS1 and HWRS2. Then 1 μl of this reaction was used for a nested PCR with primers HWRS1 and HWRS3 as above; 5 μl of the 50-μl PCR reactions was used for separation of the DNA products on a 2% agarose gel in TAE (Tris–acetate–EDTA) buffer.

Screening of a T84 cell line library

RNA was isolated from T84 cells by guanidinium thiocyanate dissolution followed by centrifugation in cesium chloride solutions; poly(A)$^+$ RNA was isolated on an oligo(dT) cellulose column (Sambrook et al., 1989). A cDNA library was constructed using the pSPORT-cDNA cloning kit (GibcoBRL). The library was screened under high-stringency conditions. The nucleotide sequence of the cDNA insert of positive clones was determined on both strands by the dideoxy chain termination method (Sanger et al., 1977). The 5′Ampli FINDER RACE kit (Clontech) with poly(A)$^+$ RNA from adult human heart (Clontech) was used to clone the remaining portion of the coding region. Specific primers from the T84A clone and from the λgt10 cloning vector sequences were also used to screen a fetal heart library (Clontech) by PCR. The full-length cDNA insert was constructed from pieces of the PCR products and pSPORT-T84 cDNA clone and subcloned into the pCDNA3 vector (Invitrogen). To verify the final construct, the pCDNA3 insert was sequenced.

Northern blot and in situ hybridization

Poly(A)$^+$ RNA from T84 cells was electrophoresed in a 1.2% agarose gel containing 2.2 *M* formaldehyde and transferred to Hybond-N membrane (Amersham). A multiple tissue northern blot containing human mRNAs (Clontech) was also used. The hybridization was performed at 42°C for 20 h in a standard solution containing 50% formamide (Sambrook et al., 1989).

A human fetal heart was obtained at autopsy after spontaneous abortion at the Centre Hospitalier Regional de Bordeaux (France) in accordance with guidelines and recommendations of the Comité National d'Ethique. The heart was fixed by immersion for 24 h in 1% paraformaldehyde, then stored at −80°C until sectioning. The *Eco*RI–*Kpn*I fragment (nucleotides 526–1,573, see Fig. 3) was subcloned into pBluescript. Radiolabeled antisense and sense cRNA probes were prepared by in vitro transcription of the pBluescript subclone with [^{33}P]UTP (Amersham). Probes were hydrolyzed to ~250 bases length and used at 20×10^6 cpm/ml. Sections (10 μm thick) were cut on a cryostat, collected on gelatin-coated slides, and fixed in 4% paraformaldehyde. Hybridization was performed as described (Brana et al., 1995) with 1 month of exposure time before development.

RESULTS

PCR amplification of a GABA receptor–related cDNA

T84 cells, derived from a colon tumor, are known to contain Cl^- channels (Schoumacher et al., 1990) and were used in reverse transcription-PCR (RT-PCR) experiments. T84 first-strand cDNA was amplified with the two degenerate oligonucleotides M2 and M3 derived from the ligand-gated chloride channel subunit transmembrane domains M2 and M3. These domains, believed to form the chloride channel pore, are conserved among the GABA/glycine receptor subunits. The PCR amplification product was cloned and the DNA sequenced. Analysis of the inferred amino acid sequence showed significant similarities with those of $GABA_A$ and glycine receptor subunits. Screening 3×10^5 clones from a T84 cDNA library with this cDNA yielded 11 positively hybridizing clones. Four clones were isolated and sequenced. Two clones, T84A (2.2 kb) and T84I (3.5 kb), contained an open reading

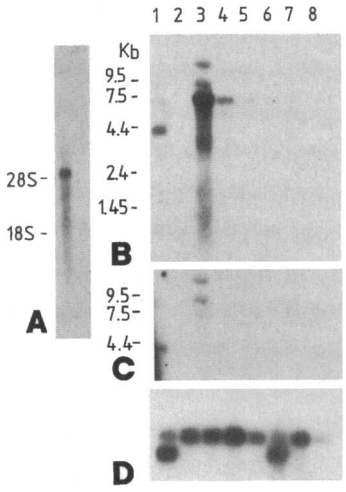

FIG. 1. Northern blot analysis of T84A $GABA_A$-like mRNA expression. Poly(A)$^+$ RNA (2 μg) was extracted from T84 cells (**A**) and from human tissues (**B, C,** and **D**). B: Heart (lane 1), brain (lane 2), placenta (lane 3), lung (lane 4), liver (lane 5), skeletal muscle (lane 6), kidney (lane 7), and pancreas (lane 8). RNAs were loaded on the gel, electrophoresed, transferred, and subjected to RNA hybridization analysis. A and B: Hybridization with the random-labeled T84A probe. C: The RNA blot shown in B was hybridized with the labeled 5′-end of the T84A clone insert. D: The RNA blot shown in B and C was hybridized with a β-actin probe to control for variation in sample loading. Molecular weights were determined using ribosomal RNA (A) or an RNA ladder (B, C).

frame (ORF) predicted to encode a protein of 242 amino acids. The other two clones, T84K and T84H, were quite similar to the T84A and T84I clones but contained an 11-bp insert (not shown) that introduced an in-frame stop codon. It is noteworthy that this insert is found at a position corresponding to a conserved intron between exons 8 and 9 of GABA and glycine receptor genes (Sommer et al., 1990). The stop codon reduced the size of the predicted protein from 242 to 116 amino acids. T84A and T84I clones were identical in their 3′-part, containing the 242 amino acids ORF; however, their 5′-part contained different sequences. The predicted 242-amino acid long protein showed significant identities with the four-transmembrane domains of GABA and glycine receptors.

To examine the tissue distribution of this mRNA, northern blots with poly(A)$^+$ RNA from T84 cells and human tissues were hybridized with a probe derived from the T84A cDNA insert (Fig. 1). T84 RNA provided a major band at 6.5 kb (Fig. 1A), migrating at the level of the 28S ribosomal RNA used as size marker. Placental RNA provided a strong signal, with a major band at 6.5 kb and minor bands at 8 and 11 kb (Fig. 1B). Hybridization was also observed with lung, liver and kidney (6.5-kb band), and heart (4-, 6.5-, and 8-kb bands) RNAs. No signal was detectable with brain, skeletal muscle, and pancreas RNAs. Northern blots using the 5′-end of the T84A insert mainly showed hybridization to heart (4-kb band) and placenta (8- and 11-kb bands) RNAs (Fig. 1C).

Identification of a novel GABA receptor-like subunit

Several independent RT-PCR amplifications were performed with human heart RNA to obtain the remaining 5′-region. A RACE-PCR procedure (Tessier et al., 1986) was used with poly(A)$^+$ RNA from adult human heart and specific primers derived from the T84A clone cDNA sequence. A fetal heart library in the λgt10 vector was also screened by PCR with specific primers from the T84A clone and from the λgt10 vector sequences. In this way, we identified a DNA sequence containing an ORF encoding a predicted protein of 505 amino acids (Fig. 2); the RT-PCR cloned part of the sequence comprises nucleotides 1–834. The cDNA contains a consensus polyadenylation signal followed by a poly(A) tail. An initiation codon was assigned based on the match to the consensus sequence for translation initiation (Kozak, 1991). Sequence analysis of the predicted protein indicates several features common to members of the GABA-gated chloride channel family. These features include an 18-residue signal peptide, a large N-terminal extracellular domain containing potential sites for glycosylation, potential transmembrane regions, and a potential protein kinase C site in the putative large intracellular region. In addition, the protein is predicted to contain a neurotransmitter-gated ion-channel signature, the Cys–Cys loop in which two cysteines separated by 13 residues are thought to participate in the formation of a disulfide bond (Sieghart, 1995) (Fig. 2).

Alignment with multiple members of $GABA_A$ and glycine receptor subunits (Pritchett et al., 1989; Ymer et al., 1989; Kuhse et al., 1990; Harvey et al., 1993; McLean et al., 1995) is shown (Fig. 3). The most conserved regions are (1) the N-terminal half, postulated to form the ligand binding domain, and (2) the transmembrane region. Pairwise alignment between the predicted protein and $GABA_A$, $GABA_C$, and glycine receptor subunits shows that this clone is most closely related to chicken GABA γ4 subunit (46% identity). The amino acid identity with mammalian subunits ranges from 40% (GABA γ2) to 25% (GABA ρ1 and glycine receptor subunits).

GABA χ mRNA expression

The regional pattern of expression of the mRNA was examined by in situ hybridization in human heart (embryonic week 20). Sections were hybridized with radiolabeled antisense cRNA specific for the coding sequence of GABA χ transcript. The mRNA is expressed exclusively and throughout the specialized electrical conduction system. Labeling is evident in the atrioventricular node, in the conduction bundle, and in the Purkinje fibers (Fig. 4). The atrioventicular node

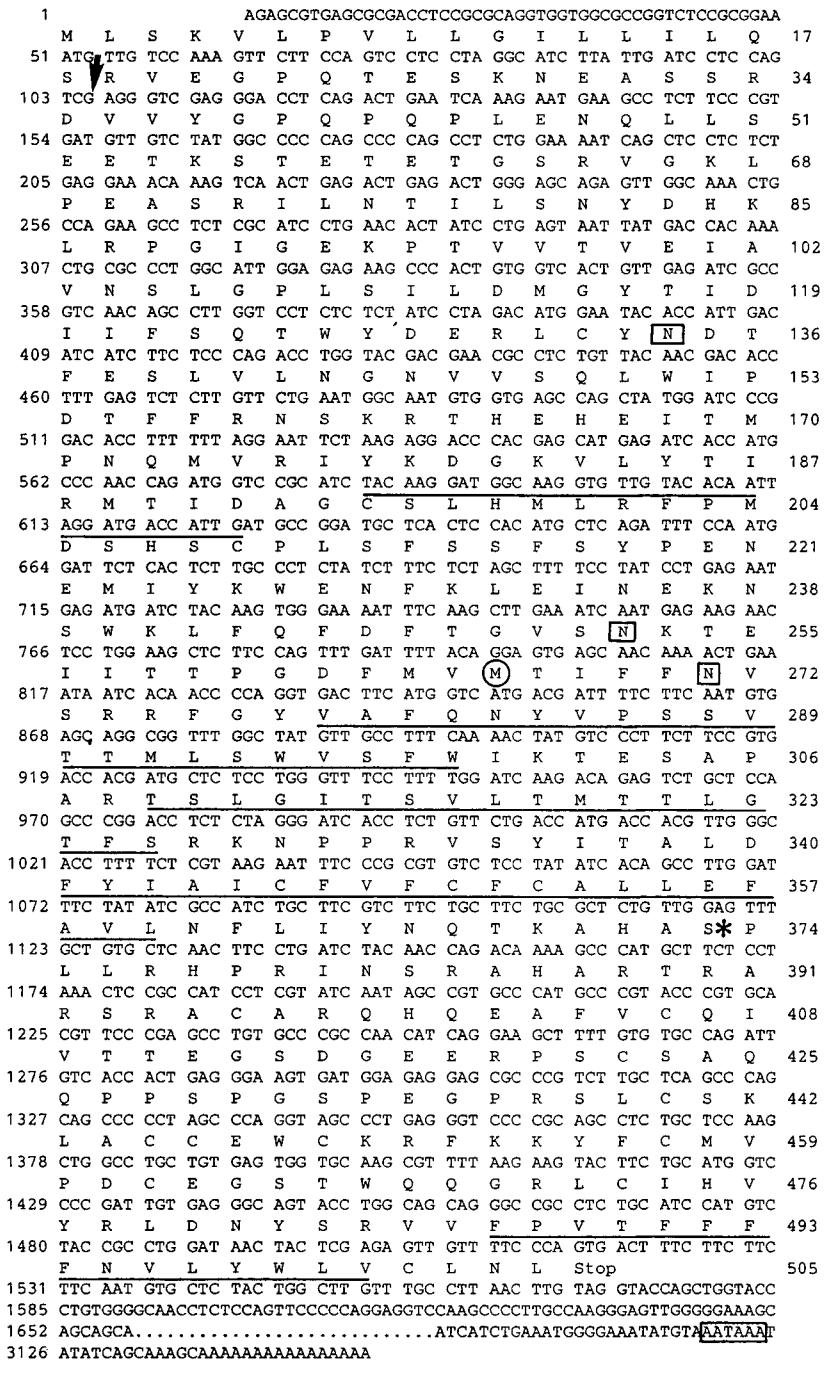

FIG. 2. Nucleotide and predicted amino acid (one-letter code) sequence of cloned cDNA indicate several features common to ligand-gated channels. The potential signal sequence cleavage site is indicated (arrow). Potential *N*-glycosylation sites are boxed; a potential protein kinase C phosphorylation site is indicated by an asterisk. The four-transmembrane segments are underlined and the ligand-gated disulfide-loop domain (amino acids 195–209) is overlined. The polyadenylation signal is boxed. The first methionine residue of the ORF encoded by T84 cDNA library clones is circled. The left column numbers refer to the first nucleotide in each line; the right column numbers refer to the last amino acid in each line. For convenience of presentation, part of the nucleotide sequence in the 3′-untranslated region corresponding to positions 1,659–3,093 was omitted. The complete cDNA sequence is available from EMBL and NCBI nucleotide sequence databases under accession number Y07637.

at the junction of the interatrial and interventricular septa (Fig. 4C) was labeled (Fig. 4A). Accumulation of silver grains was visible in the node cells (Fig. 4E). A Purkinje strand recognizable as a band of lighter staining cells under a papillary muscle and in the immediate subendocardial area (Fig. 4D) was also labeled (Fig. 4B). Accumulation of silver grains was visible in the cells underneath the endocardial cells (Fig. 4F). For each section, an adjacent section was hybridized with sense probe. As an example, Fig. 4G and H show a Purkinje strand hybridized with antisense (Fig. 4G) and sense (Fig. 4H) probes.

The sinoatrial node was not present in the heart used in these in situ hybridization experiments. This tissue was obtained after dissection of another fetal heart; total RNA was extracted and RT-PCR with GABA χ–specific primers (corresponding to nucleotide positions 148–168 and 390–415, Fig. 2) was performed. The

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glycine VLYSIRLTLTLSCPMDLKNFPMDVQTCTMQLESFGYTMNDLIFEW-LSDG-PVQVAEGLT--L 218
alpha4  ILYTMRLTISAECPMRLVDFPMDGHACPLKFGSYAYPKSEMIYTWTKGPEKSVEVPKESS-SL 222
beta2   VLYGLRITTTAACMMDLRRYPLDEQNCTLEIESYGYTTDDIEFYW-RGDDNAVTGVTKIE--L 207
gamma2  VLYSLRLTIDAECQLQLHNFPMDEHSCPLEFSSYGYPREEIVYQWKR---SSVEVGDTRSWRL 237
gamma4  VLYTLRLTIEAECLLQLQNFPMDTHSCPLVFSSYGYPREEIVYRWRR---YSIEVSDQRTWRL 220
gaba χ  VLYTIRMTIDAGCSLHMLRFPMDSHSCPLSFSSFSYPENEMIYKWEN---FKLEINEKNSWKL 242

glycine PQFILKEEKELGYCTKH--YNTGKFTCIEVKFHLERQMGYYLIQMYIPSLLIVILSWVSFWIN 279
alpha4  VQYDLIGQTVSSETIKSI---TGEYIVMTVYFHLRRKMGYFMIQTYIPCIMTVILSQVSFWIN 282
beta2   PQFSIVDYKLI---TKKVVFSTGSYPRLSLSFKLKRNIGYFILQTYMPSILITILSWVSFWIN 267
gamma2  YQFSFVGLRNTTEVVKTT---SGDYVVMSVYFDLSRRMGYFTIQTYIPCTLIVVLSWVSFWIN 297
gamma4  YQFDFTGLRNTSEVLRTG---AGEYMVMTVSFDLSRRMGYFAIQTYIPCILTVVLSWVSFWIK 280
gaba χ  FQFDFTGVSNKTEII-TT---PGDFMVMTIFFNVSRRFGYVAFQNYVPSSVTTMLSWVSFWIK 301

glycine MDAAPARVALGITTVLTMTTQSSGSRASLPKVSYVKAIDIWMAVCLLFVFAALLEYAAVNFVS 342
alpha4  KESVPARTVFGITTVLTMTTLSISARHSLPKVSYLTAMDWFIAVCFAFVFSALIEFAAVNYFT 345
beta2   YDASAARVALGITTVLTMTTINTHLRETLPKIPYVKAIDMYLMGCFVFVFMALLEYALVNYIF 330
gamma2  KDAVPARTSLGITTVLTMTTLSTIARKSLPKVSYVTAMDLFVSVCFIFVFSALVEYGTLHYFV 360
gamma4  RDSTPARTSLGITTVLTMTTLSTISRKHLPRVSYITAMDLFVSVCFIFVFAALMEYATLNYLV 343
gaba χ  TESAPARTSLGITSVLTMTTLGTFSRKNFPRVSYITALDFYIAICFVFCFCALLEFAVLNFLI 364
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FIG. 3. Comparison of predicted amino acid sequence of cloned GABA χ cDNA with human $\alpha 4$ (alpha4), $\beta 2$ (beta2), $\gamma 2$ (gamma2), and chicken $\gamma 4$ (gamma4) $GABA_A$ receptor subunit and rat $\alpha 3$ glycine receptor subunit (glycine) sequences. Only the Cys–Cys loop and M1–M3 regions are shown; amino acid numbers start with the first methionine. Identical residues in all subunits are indicated by a dot. The proposed transmembrane domains are indicated by an underbar and the consensus Cys–Cys sequence by an overbar.

expected PCR product was identified in cDNA from the sinus node and with a fetal heart cDNA library (Fig. 5). PCR amplification with specific primers for the human tryptophanyl-tRNA synthetase (Frolova et al., 1991), a ubiquitous enzyme, showed that the amount of target DNA in the two samples was comparable. We conclude that the GABA χ mRNA is also present in the sinus node.

DISCUSSION

Several types of GABA-gated Cl^- channel subunits have been characterized. These subunits can be classified into the α, β, γ, δ, and ρ subfamilies according to their amino acid sequence. Members of the same class exhibit 70–80% amino acid sequence identity, compared with 30–40% identity between different classes. The newly found subunit reported here shares 45–25% amino acid sequence identity with the $GABA_A$ and $GABA_C$ receptor subunits and may therefore represent a novel subfamily of the GABA-gated Cl^- channels. We propose to name this clone and putative subfamily GABA χ.

We have introduced The GABA χ clone into CHO cells, COS cells, and *Xenopus* oocytes (M. Garret, unpublished data). However, GABA and glycine failed to activate whole-cell currents in transfected cells. Two GABA binding domains have been identified by mutagenesis on GABA $\beta 2$ receptor subunit (Amin and Weiss, 1993; amino acids 181–184 and 226–229, Fig. 3). The GABA χ-predicted polypeptide does not contain the same sequence at these positions (amino acids 216–219 and 260–263, Fig. 3). This difference may explain the insensitivity of the GABA χ-transfected cells to GABA. However, previous studies of single $GABA_A$ receptor subunits expressed by cell transfection either did not detect any current or noted small currents activated only by high concentrations of GABA (Schofield et al., 1987). Robust GABA-gated Cl^- currents are found only after coexpression of different types of GABA receptor subunits. The GABA χ subunit may also be part of a heteromultimeric receptor. Coexpression of this clone with other members of the $GABA_A$ receptor subunit family or analysis of the GABA χ polypeptide with antibodies may answer this question. However, it is also possible that this clone encodes a receptor for an unidentified ligand.

The DNA sequence of clones obtained from the T84 cDNA library and the northern blot experiments indicate that an unspliced form of GABA χ mRNA is present in some human tissues. The presence of an unspliced intron in $\alpha 3$ $GABA_A$ receptor subunit mRNA has been reported (Levitan et al., 1988). Incompletely or aberrantly spliced mRNAs have also been noted for the $GABA_C$ $\rho 2$ subunit in human retina (Cutting et al., 1992). For some $GABA_A$ receptor subunits, northern blot experiments with RNA extracted from several brain regions revealed the presence of multiple bands, suggesting alternative splicing of these subunits (Khrestchatisky et al., 1991; O'Hara et al., 1995). Moreover, the gene that encodes the GABA $\beta 3$ subunit contains two alternative 5′-untranslated sequences and a strong promoter (Kirkness and Fraser, 1993). RNase protection showed that mRNA for this subunit is widely expressed, including immortalized cell lines such as human kidney 293 cells (Kirkness and Fraser, 1993). It would be interesting if mRNA for this $\beta 3$ subunit (or others) is also expressed, spliced or not, in tissues outside the CNS.

Recent evidence indicates that GABA may have diverse functions other than neurotransmission in peripheral tissues such as the gut, urinary bladder, lung, ovary, heart, and pancreas (for review, see Erdö and Wolff, 1990). GABA-gated chloride channels are present in pancreatic and pituitary cells (Anderson and Mitchell, 1986; von Blankenfeld et al., 1995) where

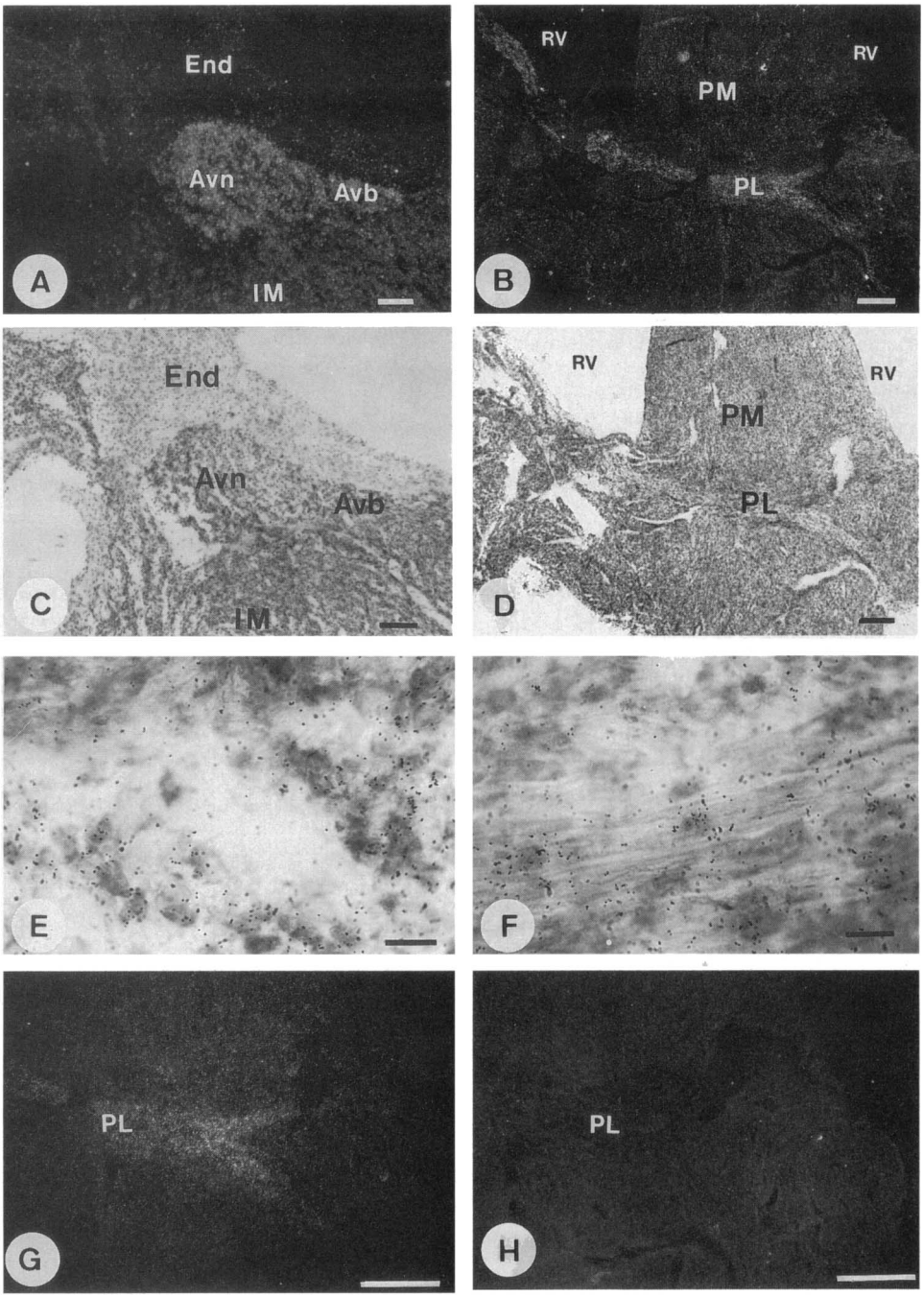

FIG. 4. Regional expression of GABA χ subunit in a horizontal section of fetal human heart. Avn, atrioventricular node; Avb, atrioventricular bundle; IM, interventricular myocardium; End, endocardium; RV, right ventricle; PM, papillary muscle; PL, Purkinje layer. **A** and **B:** Dark-field photomicrographs of sections hybridized with a ^{33}P-labeled probe. **C** and **D:** Bright-field photomicrographs of Coomassie Blue–stained sections are shown for comparison. **E** and **F:** High-resolution bright-field microscopy of Coomassie Blue–stained sections showing silver grains (black dots) indicative of probe hybridization. **G** and **H:** Dark-field photomicrographs of adjacent sections hybridized with antisense (G) and sense (H) probes. The scale bars represent 200 μm (A, B, C, D, G, and H) and 10 μm (E and F).

they regulate hormone secretion. These channels appear to be identical to those found in the CNS (Boué-Grabot et al., 1995). If the sequence reported here does indeed encode a GABA-gated chloride channel, this would be the first case of such a subunit specifically expressed outside of the CNS.

The peripheral and central GABAergic systems affect the regulation of cardiovascular function (Matsu-

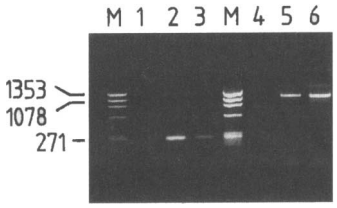

FIG. 5. Expression of GABA χ mRNA in sinus node. This figure shows ethidium bromide-stained products of PCR amplifications resolved by electrophoresis in 2% agarose gel. Oligonucleotide primers specific for GABA χ subunit (lanes 1–3) and for the ubiquitous tryptophanyl-tRNA synthetase (lanes 4–6) were used. PCRs were performed on cDNA obtained from fetal sinus node (lanes 2 and 5) or on adult heart cDNA library (lanes 3 and 6). Control experiments were run with water instead of DNA (lanes 1 and 4). Standard molecular weight markers (M) are *Hae*III cut Φ X174. The positions of 1,353, 1,078, and 271–281 nucleotide bands are indicated to the left of the gel.

yama et al., 1991). Among various regions of the guinea pig heart, it has been shown that the amount of GABA is highest in the sinus node, where it may act through a $GABA_A$ receptor located on cell bodies of the postganglionic cholinergic neurons (Matsuyama et al., 1993). Our finding opens the possibility that GABA acts directly on cardiac function through GABA receptors located within the electrical conduction system cells. The cardiac conduction system is responsible for the initiation and the propagation of the heartbeat and expresses both muscle- and neuron-specific genes. Both myogenic and neural origins have been suggested for this specialized tissue (Gorza et al., 1988; Gourdie et al., 1995). The expression of an mRNA encoding a protein similar to a neurotransmitter receptor is consistent with the neuronal properties for these cardiac cells (Gorza et al., 1988). Our northern blot, PCR, and in situ hybridization experiments on adult and fetal heart suggest that GABA χ mRNA is expressed at all stages of development. This may be relevant to function and dysfunction of this system.

Note added in proof: While this article was in press, the same mRNA was found expressed in the brain subthalamic nucleus. This subunit named ϵ can form a GABA-gated chloride channel when coexpressed in the presence of both an α and a β $GABA_A$ receptor subunit [Davies P. A., Hanna M. C., Hales T. G., and Kirkness E. F. (1997) Insensitivity to anaesthetic agents conferred by a class of $GABA_A$ receptor subunits. *Nature* **385,** 820–823].

Acknowledgment: This study was supported by CNRS, the University of Bordeaux II, and the region Aquitaine. We thank all the members of the Centre Hospitalier Regional de Bordeaux, especially Dr. D. Cardes. We thank A. R. Marks for comments on the manuscript.

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