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Functional Characteristics and Sites of Gene Expression of the $\alpha_1,\beta_1,\gamma_2$-Isoform of the Rat $GABA_A$ Receptor

P. Malherbe,[1] E. Sigel,[2] R. Baur,[2] E. Persohn,[1] J. G. Richards,[1] and H. Mohler[3]

[1]Pharmaceutical Research Department, Hoffmann-La Roche AG, Basel, Switzerland, [2]Institute of Pharmacology, University of Bern, Switzerland, and [3]Institute of Pharmacology, University of Zurich, Switzerland

$GABA_A$ receptors, the major synaptic targets for the neurotransmitter GABA, constitute gated chloride channels. By their allosteric, drug-induced modulation, they serve as control elements for the regulation of anxiety, vigilance, and epileptiform activity. The structural requirements of fully functional $GABA_A$ receptors in the mammalian brain have remained elusive so far. We report here on the cloning of the γ_2-subunit cDNA of rat brain and its functional analysis by coexpression with the α_1- and β_1-subunits in *Xenopus* oocytes, and on the sites of gene expression of the 3 subunits in the rat brain. The recombinant receptor displayed GABA-inducible currents ($I_{max} = 6\mu A$; $K_a = 75\ \mu M$) which were allosterically modulated by benzodiazepine receptor ligands (enhancement and inhibition by diazepam and methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate, respectively). In the absence of GABA, pentobarbital elicited a maximal current amplitude similar to that of GABA. A minor population of channels is expressed which is open in the absence of GABA or pentobarbital. Mapping subunit gene expression by *in situ* hybridization histochemistry suggests that the α_1-, β_1-, and γ_2-subunits are likely receptor constituents in some neuronal populations, e.g., mitral cells of the olfactory bulb, pyramidal cells of the hippocampus, and granule cells of the dentate gyrus and cerebellum.

The $GABA_A$ receptor, the major target for the neurotransmitter GABA in the CNS, constitutes a gated chloride channel whose allosteric modulation by drugs is exploited therapeutically in the treatment of anxiety, insomnia, muscle spasms, and epilepsy. Following the initial identification of α- and β-subunit proteins as constitutive components of the receptor (Möhler et al., 1980; Sigel et al., 1983; Sigel and Barnard, 1984; Schoch et al., 1984), cloning and functional expression of subunit cDNAs was expected to yield further information on the structural requirements of the native $GABA_A$ receptor. The reconstitution of GABA-gated chloride channels was first achieved by expressing in *Xenopus* oocytes the cDNAs coding for the α_1- and β_1-subunits of bovine, human, and rat brain (Schofield et al., 1987, 1989; Mahlerbe et al., 1990). However, even when the α_1-subunit was replaced by other α-subunit variants (Levitan et al., 1988a), the recombinant $GABA_A$ receptors lacked major functional characteristics. While 2 molecules of GABA are needed for the channel to open *in vivo*, there was no evidence for GABA cooperativity in the recombinant receptor of the bovine or human brain (Levitan et al., 1988a, b; Pritchett et al., 1988), although some cooperativity was found in the recombinant receptor of the rat brain (Malherbe et al., 1990). Furthermore, the bidirectional modulation of the GABA response by agonists and inverse agonists, respectively, of the benzodiazepine receptor (BZR) was either absent (Levitan et al., 1988a, b; Pritchett et al., 1988) or functionally incomplete (Malherbe et al., 1990). The ability to mediate both the positive and negative intrinsic activity of BZR ligands appears to depend on the presence of the γ_2-subunit recently cloned from a human brain cDNA library (Pritchett et al., 1989). When the cDNAs coding for the human α_1- and β_1-subunits were coexpressed with that of the γ_2-subunit, a fully functional BZR site became apparent (Pritchett et al., 1989); the GABA response was enhanced by diazepam and reduced by methyl-6,7,dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM) in a flumazenil-sensitive manner. However, no evidence for cooperativity of GABA was found in the recombinant receptor expressed from human α_1-, β_1-, and γ_2-subunits.

In order to further investigate the functional potential of the γ_2-subunit, the corresponding cDNA was isolated from rat brain tissue and coexpressed in *Xenopus* oocytes with the rat α_1-, and β_1 subunit cDNAs. In addition, we investigated whether the α_1-, β_1-, and γ_2-subunits represent a likely subunit combination in the rat brain *in vivo*. The pattern of gene expression of the γ_2-subunit was visualized by *in situ* hybridization histochemistry and compared to the pattern of gene expression of the α_1- and β_1-subunits.

Materials and Methods

Isolation of the γ_2-subunit cDNA clone. Following the construction of a cDNA library from 7-d-old rat brain mRNA in the λgt11 vector as reported (Malherbe et al., 1990), 1×10^6 recombinant transformants were screened with a ^{32}P-nick-translated rat β_1-subunit cDNA probe [a 250 base pair (bp) EcoRI-EcoRV fragment]. The hybridization filters were washed in 2 × SSC (standard saline citrate), 0.1% SDS at 50°C (1 × SSC = 0.15 M NaCl:0.015 M sodium citrate). The weakly hybridizing clone λOT2γF23 was subcloned into an M13 vector and sequenced by the chain termination method (Sanger et al., 1980).

Preparation of cRNA for oocyte injection. Fragments of the rat α_1-, β_1-, and γ_2-subunit cDNAs (α_1, 1503 bp; β_1, 1941 bp; γ_2, 3800 bp) were inserted at the EcoRI site into the plasmid pSpT19 (Pharmacia) for transcription. The cRNA was capped and polyadenylated as previously described for the α_1- and β_1-subunit cDNAs (Malherbe et al., 1990).

Expression of the cloned subunits in the Xenopus *oocyte.* Follicle cells were surgically removed from female *Xenopus laevis*, mechanically isolated, maintained in culture, and, on the following day, microinjected with about 50 nl of a solution containing the capped transcripts coding for each of the different subunits at a concentration of 200 nM. All

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Correspondence should be addressed to Dr. H. Mohler, Institute of Pharmacology, University of Zurich, Gloriastr. 32, 8006 Zurich, Switzerland.

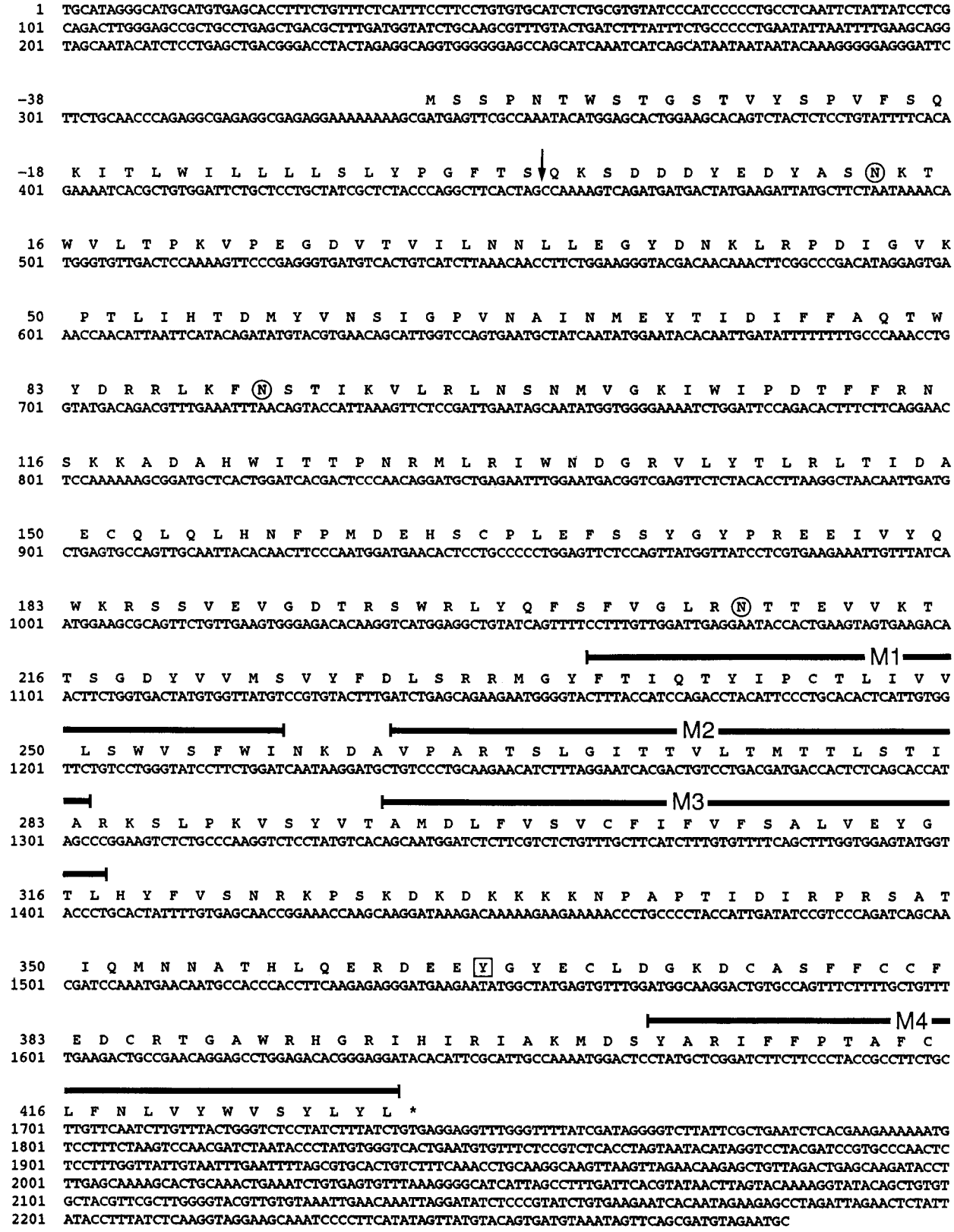

Figure 1. Nucleotide and deduced amino acid sequence of the γ_2-subunit cDNA of the $GABA_A$- receptor from the rat brain. Potential sites of *N*-linked glycosylation are *circled.* The proposed membrane-spanning regions (M1–M4) are indicated by *solid lines.* The consensus site for tyrosine phosphorylation is *boxed.* The *arrow* points to the putative signal sequence cleavage site. The sequence shown (2282 bases) was derived from a 3800 bases cDNA clone.

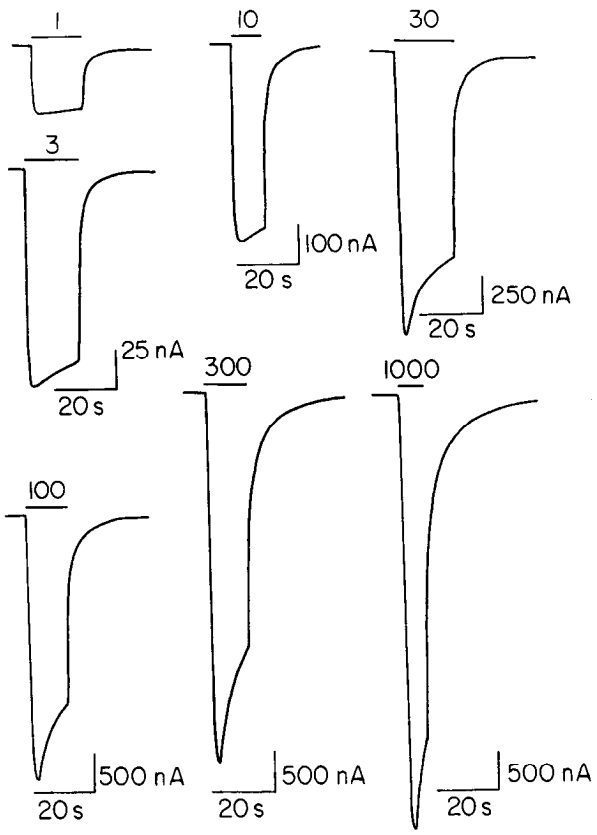

Figure 2. Membrane current response to different concentrations of GABA. The oocyte was kept at −80 mV under voltage-clamp. Continuous perfusion was switched for the periods of time indicated by the *bar* to the same medium containing GABA. Concentrations are indicated in μM.

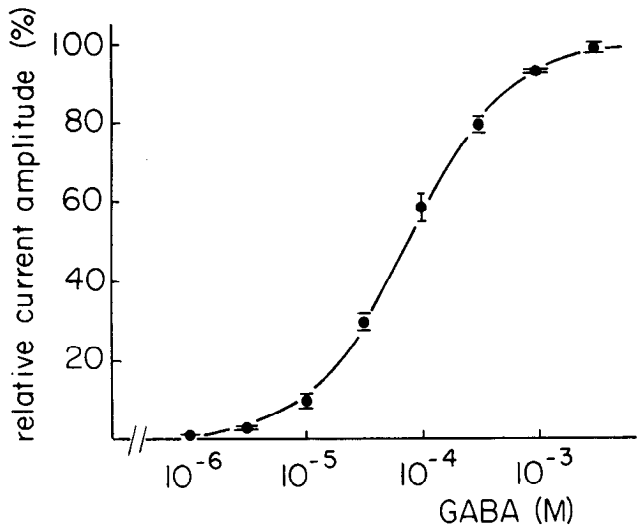

Figure 3. Concentration dependence of the GABA-induced current response. The dose–response curve was recorded 3 times from different oocytes, with cells exhibiting different degrees of channel expression. Each curve was fitted by Equation (1). Maximal current was then arbitrarily set at 100%, and the current amplitudes were expressed in relative values. Each *point* represents the average of 3 determinations. The *curve* shown was obtained by reapplying the fit to the standardized, averaged values.

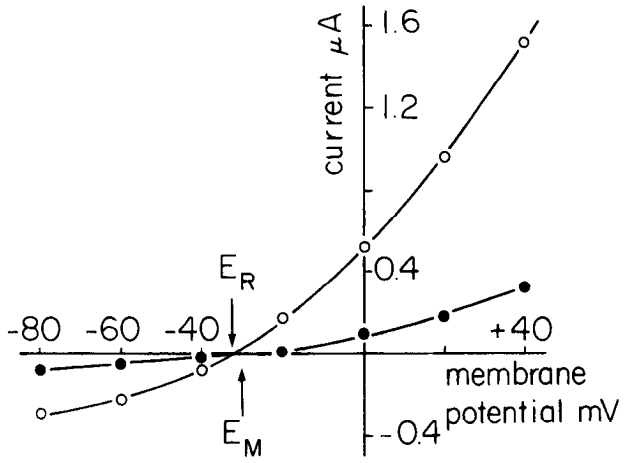

Figure 4. Current voltage relationship of the GABA response. Before and during GABA application, a discontinuous voltage ramp of 100 msec duration was applied from a holding potential of −80 mV. The current amplitude measured at the end of the step was plotted against the membrane potential. ●, resting conditions; ○, 10 μM GABA; E_M, membrane potential; E_R, reversal potential.

electrophysiological measurements were carried out on denuded oocytes. The follicular cell layers were removed as described (Sigel, 1987), one day before the experiments. Oocytes were placed on a nylon grid in a 0.4 ml bath, which was perfused throughout the experiment at 6 ml/min with 90 mM NaCl: 1 mM KCl: 1 mM $MgCl_2$: 1 mM $CaCl_2$: 5 mM HEPES-NaOH (pH 7.4). The perfusion medium could be switched to one supplied with GABA alone or GABA in combination with other drugs and was applied through a glass capillary with an inner diameter of 1.35 mm. The tip of the capillary was placed about 0.4 mm from the surface of the oocyte to allow fast changes in ligand concentration around the oocyte. The rate of medium exchange amounted to 70% in less than 0.5 sec, as determined by switching the perfusion medium to a sodium-free medium and measuring the amplitude of Na currents expressed in oocytes injected with cRNA isolated from chick forebrain. All experiments were carried out at room temperature (22–26°C). For the current measurements, the oocytes were impaled with 2 microelectrodes, and the membrane potential was voltage-clamped at −100 mV as described (Sigel, 1987). Diazepam was diluted into standard medium from preparations used for intravenous application (Valium, 17.6 mM in H_2O/propylene glycol/ethanol/Na-benzoate/benzyl alcohol/benzoic acid). DMCM was obtained from Research Biochemicals (Konstanz, West Germany) and diluted from an ethanol solution. The final concentration of ethanol in the experiment was 0.06%. Neither of the drug carriers altered the GABA response in control experiments. Current amplitudes were read as the peak currents. Dose response curves were fitted using a nonlinear least-squares method (Gauss-Newton-Marquardt).

In situ *hybridization histochemistry.* The cRNA probes were labeled, and the hybridization procedure was performed as previously described for the α_1- and β_1-subunits (Séquier et al., 1988). Briefly, formaldehyde-fixed, 10 μM cryostat sections of rat brain were washed with a prehybridization buffer, dehydrated, then hybridized with ^{35}S-labeled, single-stranded RNA (antisense and sense) transcripts at 60°C for 20 hr. Sections were then rinsed, washed finally in 1 × SSC at 75°C for 1.5 hr, dehydrated, exposed to sheet film (Hyperfilm, β-Max, Amersham), and then dipped in a nuclear track emulsion (K5, Ilford) to reveal the regional and cellular localization of the α_1, β_1, and γ_2 probes, respectively.

Results

Cloning of the γ_2-subunit cDNA from the rat brain

Screening of a cDNA library of rat brain with a rat β_1 cDNA probe encoding the M1 and M2 transmembrane domains at low stringency resulted in the isolation of various clones with different hybridization intensities. One of the most weakly hybridizing clones, λOT2γF23 (about 3800 bp), contained a 5′

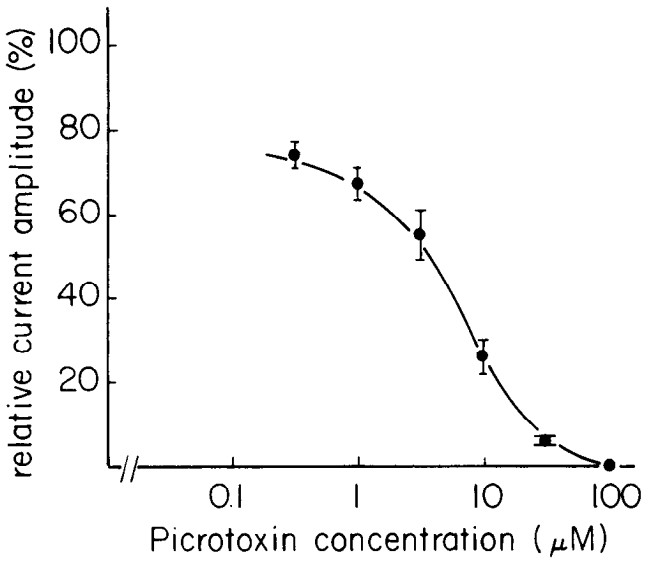

Figure 5. Inhibition of the GABA-induced current by picrotoxin. GABA (10 μM) was applied to the oocyte, first alone and then in combination with increasing concentrations of picrotoxin. Data from 3 experiments were averaged after standardization of the control response in the absence of picrotoxin to 100%.

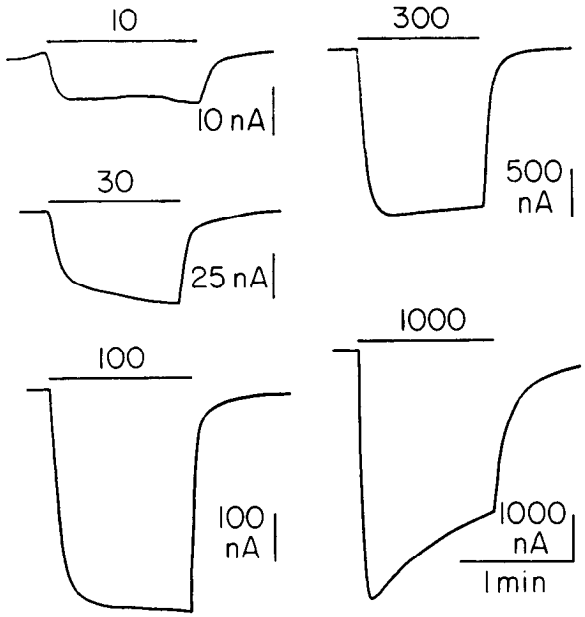

Figure 6. Pentobarbital elicits large inward currents in the absence of GABA. Experimental conditions were similar as described for Figure 2, except that continuous perfusion was switched to a medium containing pentobarbital (*bar*). Concentrations are indicated in μM.

noncoding sequence of 342 nucleotides in a nucleotide sequence of 2282 bp, which was followed by an open reading frame of 1739 nucleotides encoding a 428-amino acid polypeptide (Fig. 1). The deduced amino acid sequence is highly similar (98.7%) to that of the human γ_2-subunit sequence (Pritchett et al., 1989), differing only in the putative signal peptide by 2 substitutions and 1 deletion [I(−34) → T(−33), T(−29) → S(−28), T(−24)] and by 2 substitutions in the extracellular region [M(81) → T(81), S(142) → T (142)]. The overall sequence similarity between the rat γ_2-subunit and the α_1- and β_1-subunits of the rat brain (Lolait et al., 1989; Ymer et al., 1989; Malherbe et al., 1990) is 44% and 37%, respectively. The predicted primary structure of the γ_2-subunit exhibits many features similar to the rat α_1- and β_1-subunits. A hydrophobic amino terminal sequence of 38 amino acids indicates a signal peptide (von Heijne, 1986). A disulphide-bonded loop [cysteine (Cys) 151 to Cys 165] and 3 putative *N*-glycosylation sites [asparagine (Asn) 13, Asn 90, and Asn 208] are found in the presumed extracellular domain. Four hydrophobic domains with predicted secondary structure may span the membrane (M1–M4). The presumed cytoplasmic loop connecting the transmembrane domains M3 and M4 contains a consensus site for tyrosine-specific protein phosphorylation (tryosine 365) (Hopfield et al., 1988). This site may play a role in the regulation of neuronal signal transduction. A rat γ_2-subunit sequence identical to the one shown here was published (Shivers et al., 1989) since submission of this manuscript.

Functional expression of the α_1-, β_1-, and γ_2-subunits

In order to test the functional properties of the rat γ_2-subunit, oocytes were injected with a combination of cRNAs coding for the α_1-, β_1-, and γ_2-subunits of the rat brain. While noninjected oocytes were never observed to respond to GABA, the injected oocytes displayed large inward currents when superfused with GABA. At 100 μM GABA, the peak current amplitude was 3.9 ± 1.9 μA (mean ± SD of 33 oocytes from 4 different injection experiments performed with different batches of oocytes). The GABA-induced current was concentration dependent, as shown in Figure 2. Traces were recorded upon applying increasing concentrations of GABA to a single oocyte. Because the current response showed densensitization at higher GABA concentrations, the recording of the traces was interrupted for periods of time sufficient to allow for recovery from desensitization. A summary of 3 such experiments performed on 3 different oocytes is given in Figure 3. Individual dose–response curves were fitted (see Materials and Methods) using the equation

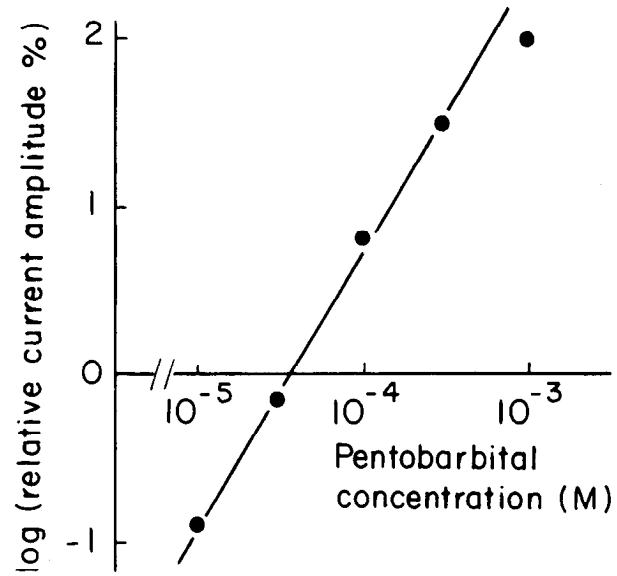

Figure 7. Log-log plot of the pentobarbital dose–response curve. Data from 3 experiments were averaged after standardization to 100% of the current responses elicited by 1 mM pentobarbital, in order to correct for differential extent of current expression in the different oocytes.

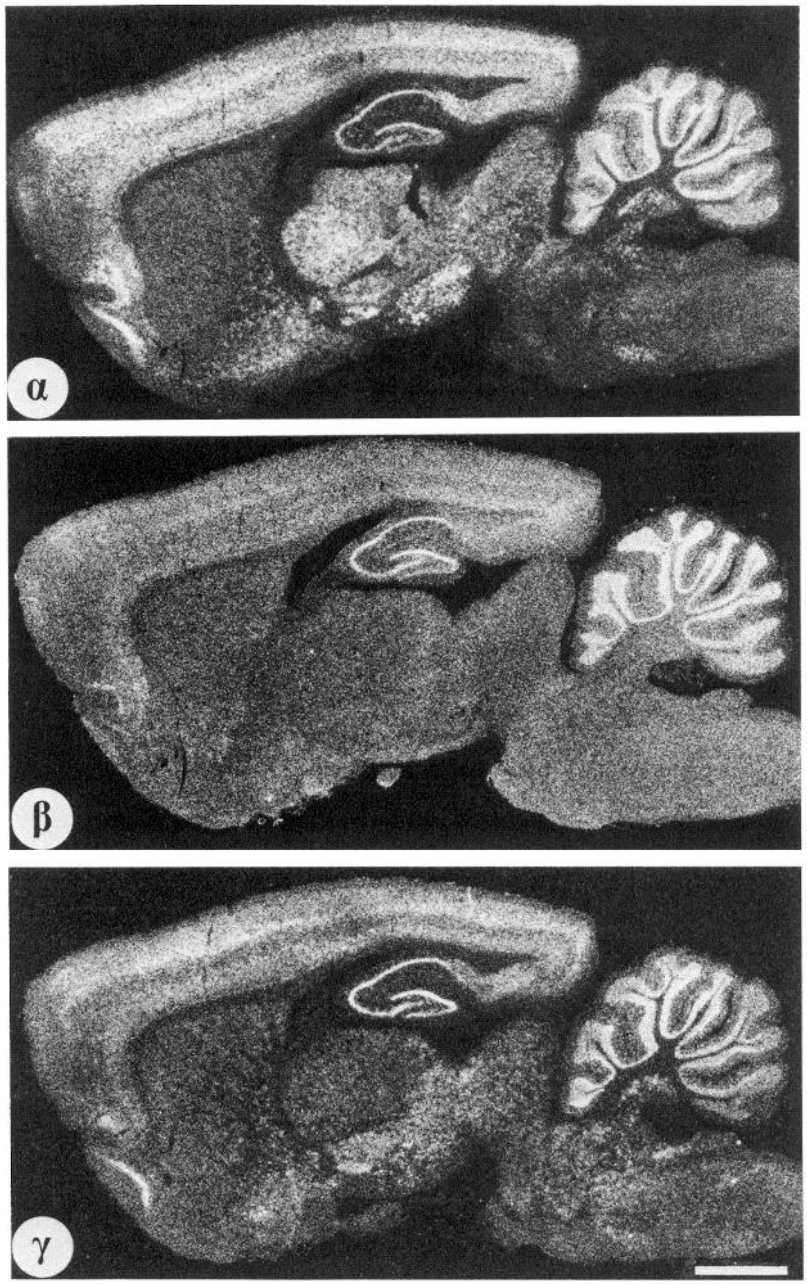

Figure 8. *In situ* hybridization of the ^{35}S-labeled RNA antisense strand of the γ_2-subunit (*bottom*) and its comparison with that of α_1- (*top*) and β_1-subunits (*middle*) in parasagittal adjacent sections of rat brain. *White areas* correspond to brain regions with an intense signal. Scale bar, 2 mm.

$$I(c) = I_{max}c^n/(c^n + K_a^n), \quad (1)$$

where I_{max} is the maximal current amplitude, c is the GABA concentration, n is the Hill coefficient, and K_a is the GABA concentration that elicits a half-maximal current response. Individual curves were standardized by setting I_{max} = 100%, and the 3 experiments were then averaged and fitted again with Equation (1) to give the solid line in Figure 3. The best fit was obtained with K_a = 75 μM and n = 1.03. The averaged I_{max} before standardization was 6 μA. In the present experiments, a fast ligand application technique was used in order to minimize errors introduced by desensitization in the determination of the current amplitudes. Back-extrapolation of the desensitizing current traces to the time when the current reached 50% of the maximal amplitude indicated that this error did not exceed 25% at the highest ligand concentrations used. Desensitization on a time scale faster than hundreds of msec would be missed with the present techniques. The Hill coefficient was derived from the data by evaluating the limiting slope of the log-log plots (data not shown). This method, which relies on the data obtained at low agonist concentrations where desensitization is slow, yielded a Hill coefficient of 1.06. The observed kinetic parameters have to be viewed in comparison to those obtained with oocytes injected with total chick brain mRNA, where similar experiments resulted in I_{max} of about 1.5 μA, K_a = 21 μM, and n = 1.69 (Sigel and Baur, 1988).

Voltage dependence of the GABA currents

The voltage dependence of the GABA response (Fig. 4) was similar to that described earlier (Miledi et al., 1982; Sigel and Baur, 1988) for oocytes injected with chick brain mRNA. The reversal potential was −35 ± 4 mV (5 experiments), a value

slightly more negative than the normal chloride equilibrium potential in the *Xenopus* oocyte (Barish, 1983). This may be due to the expression of a small percentage of channels open in the absence of GABA (see below). This form of channel could result in a loss of intracellular chloride. Alternatively, the channel formed could have an altered ion selectivity.

Modulation of the GABA current by drugs

The effects of positive and negative allosteric modulators that act at the benzodiazepine binding site were tested on the GABA-gated channels expressed from α_1-, β_1-, and γ_2-subunit cRNAs. We found small but significant effects by these modulators, indicating that a benzodiazepine binding site was present on the recombinant receptors. The current amplitude elicited by 2.5 μM GABA was enhanced by 17 ± 7% (mean ± SD; $n = 11$) in the presence of 1 μM diazepam and decreased by 6 ± 4% (mean ± SD; $n = 6$) in the presence of 0.3 μM DMCM ($p < 0.01$; Student paired t-test). The drug concentrations were chosen based on earlier experiments with the GABA receptor channel expressed from total chick forebrain mRNA, where these concentrations caused maximal modulation of the GABA current (Sigel and Baur, 1988). Qualitatively, the modulatory effects observed at the recombinant receptor were in line with the intrinsic activity of the drugs *in vivo*. Quantitatively, however, the degree of modulation is at least 6-fold smaller than that found previously with the same agents on the GABA receptors expressed from total chick forebrain mRNA (Sigel and Baur, 1988).

Glycine (1 mM) elicited small inward currents amounting to less than 0.5% of I_{max} (4 experiments) induced by GABA. Picrotoxin, applied in the absence of GABA, caused a small shift in the holding current in a direction opposite to the current elicited by GABA. This small shift amounted to less than 0.5% of I_{max} (20 ± 6 nA; 6 experiments) and presumably indicates that a minor fraction of channels is open in the absence of added GABA. This is reminiscent of the nongated, picrotoxin-sensitive anion channel observed in oocytes injected with the β_1-subunit cRNA only (Sigel et al., 1989). When applied in the presence of GABA, picrotoxin dose-dependently inhibited the GABA-induced current (Fig. 5). The shape of the inhibition curve was not monophasic, indicating a complex interaction of picrotoxin with the channel. Half-maximal inhibition was observed at about 6 μM. This is about a 10-fold increase over the concentration needed for half-maximal inhibition of the GABA channel expressed from total chick forebrain mRNA (Sigel and Baur, 1988).

Maximum activation of the channel by pentobarbital in the absence of GABA

Moderate concentrations of pentobarbital (10-1000 μM) elicited inward currents in the absence of GABA (Fig. 6). These currents had a reversal potential of −37 ± 2 mV (2 experiments), very similar to the reversal potential of GABA-induced currents. At pentobarbital concentrations higher than 1 mM, the currents diminished (Fig. 6), which may be due either to a rapid desensitization or to a secondary inhibitory effect. From a series of dose–response experiments, pentobarbital was estimated to elicit a half-maximal current response at a concentration of about 400 μM. From the log-log plot of the dose–response curve (Fig. 7), a limiting slope of 1.6 was deduced, indicating cooperativity of pentobarbital in gating the channel. The maximal current amplitude elicited by pentobarbital (1 mM) was similar to the maximal amplitude induced by GABA (3 mM). Thus, the receptor expressed from the α_1-, β_1-, and γ_2-subunit cRNAs can be fully activated by pentobarbital. This is in contrast to experiments performed previously on *Xenopus* oocytes with GABA channels induced by total rat forebrain mRNA, where 1 mM pentobarbital activated only very small currents as compared to the I_{max} in response to GABA (Parker et al., 1986).

Table 1. Regional expression of the α_1-, β_1- and γ_2-subunit genes in the rat brain

Brain region	α_1	β_1	γ_2
Olfactory bulb mitral cells	+++	+	++
Cerebral cortex			
lamina I	0	0	0
lamina II + III	+	+	(+)
lamina IV	0	0	0
lamina V + VI	+	+	(+)
Pallidum			
ventral pallidum	++	0	(+)
globus pallidus	++	0	(+)
insulae Calleja	++	0	0
Hippocampal formation			
cells in stratum oriens	+	0	0
pyramidal cells CA1-3	+	+	+
presumptive interneurons	++	+	+
dentate gyrus	+	+	++
subiculum	+	0	(+)
Thalamus			
anterior pretectal nucleus	++	0	+
lateral posterior nucleus	++	0	+
laterodorsal nucleus	++	0	0
ventral posteromedial nucleus	++	0	0
ventrolateral nucleus	++	0	0
subthalamic nucleus	++	0	+
zona incerta	++	0	+
Substantia nigra (zona reticularis)	+++	0	(+)
Cerebellum			
molecular layer	+	0	(+)
Purkinje cells	+++	0	++
granular layer	+	(+)	+
Colliculus inferior	++	0	+
Subbrachial nucleus	+++	0	+
Nucleus interpositus	+++	0	+
Vestibular nucleus	+	0	+
Facial nucleus	++	0	+

In situ hybridization signals obtained with ^{35}S-labeled cRNA probes on serial sections were qualified as intense, +++; strongly positive, ++; positive, +; weakly positive, (+); or not detectable, 0.

Sites of subunit gene expression

The γ_2-subunit appears to be widely expressed in rat brains, as shown by *in situ* hybridization histochemistry using the ^{35}S-labeled γ_2-cRNA antisense strand. A positive signal was visualized in major brain areas with, however, noticeable exceptions; in particular, some thalamic nuclei, lamina I and IV in the cerebral cortex, and the islands of Calleja (Fig. 8, Table 1). The hybridization signal of the γ_2-cRNA probe does not appear to be due to cross-hybridization with the α_1- or β_1-mRNA in the tissue, as shown by the lack of the γ_2-signal in areas which show a strong α_1-signal (e.g., some thalamic nuclei) and by the presence of the γ_2-signal in the absence of a β_1-signal (e.g., in sub-

stantia nigra). Furthermore, because the overall nucleotide sequence identity of the γ_2-cDNA with the α_1- and β_1-cDNA is only 55% and 51%, respectively, a cross-hybridization would not be expected at the hybridization and washing temperature used (Séquier et al., 1988).

In order to compare the pattern of gene expression of the γ_2-subunit with that of the α_1- and β_1-subunits, *in situ* hybridization histochemistry was performed with the respective cRNA probes on serial sections of rat brain (Fig. 8, Table 1). While only background labeling was observed with the sense-cRNA strands of all 3 subunits, the antisense-strands yielded strong hybridization signals. The α_1-signal was clearly the most ubiquitous and intense. Coexpression of all 3 subunits was apparent in several brain regions, including the cerebral cortex, olfactory bulb (mitral cell layer), hippocampal formation (pyramidal and granule cells), and cerebellum (granule cells). However, in some brain regions (subiculum; pallidum; zona incerta; substantia nigra; cerebellar Purkinje cells and molecular layer; colliculus inferior; subbrachial nucleus; nucleus interpositus; vestibular and facial nuclei; certain thalamic nuclei: anterior pretectal, lateral posterior, subthalamic), only α_1- and γ_2-transcripts were detected. In other regions, only α_1-transcripts were detected (hippocampal nonpyramidal cells in the striatum oriens; certain thalamic nuclei: laterodorsal, ventral posteromedial, ventrolateral). The hybridization patterns obtained with the cRNA strands were reproduced with labeled oligonucleotide probes specific for either the α_1-, β_1-, or γ_2-subunit cDNAs (J. G. Richards, unpublished observations). This underlines the subunit specificity of the cRNA hybridization pattern.

Discussion

The recombinant receptor expressed in *Xenopus* oocytes from α_1-, β_1-, and γ_2-subunit cRNAs of the rat brain qualitatively shares major properties with the native $GABA_A$ receptor and with the receptor expressed in *Xenopus* oocytes from total brain polyA$^+$ RNA. In particular, a bidirectional modulation of the GABA response by positive and negative allosteric modulators acting at the BZR could be observed. However, the extent of the modulation of the GABA response by diazepam and DMCM was 6 times smaller than that observed at the same drug concentrations in *Xenopus* oocytes injected with total chick brain RNA (Sigel and Baur, 1988). This low modulatory response does not seem to be due to the absence of a yet unknown subunit because the recombinant human α_1-, β_1-, γ_2-receptor expressed in mammalian cells displayed a modulatory response to diazepam and DMCM, which is comparable to findings *in situ* (Pritchett et al., 1989). Thus, differences in protein processing or in protein-lipid interactions may contribute to these differences of recombinant receptors expressed in the 2-expression systems. Furthermore, differences between human and rat clone expression rates and RNA stabilities may lead to differences in subunit stoichiometry. Alternatively, structural differences due to nonconservative amino acid substitutions or deletions in the extracellular domains of the human and rat subunits may be contributory factors. The human γ_2-subunit contains methionine (81) instead of threonine (81), the human β_1-subunit serine (10) instead of proline (10), and the human α_1-subunit phenylalanine (65), arginine (95), and leucine (4) instead of leucine (65), tryptophane (95), and a deletion (4). However, it cannot be excluded that additional subunit variants may be necessary to provide a fully functional receptor isoform of rat brain.

Compared to the receptor expressed from total chick brain polyA$^+$ RNA in *Xenopus* oocytes, the recombinant $\alpha_1,\beta_1,\gamma_2$-receptor displays roughly a 10-fold reduced potency of picrotoxin to inhibit the GABA response. Furthermore, the ability of high concentrations of pentobarbital to directly activate the channel is much more pronounced in the $\alpha_1,\beta_1,\gamma_2$-receptor, where pentobarbital induces the same I_{max} as GABA, than in oocytes injected with total RNA, where only 5% of the GABA-induced I_{max} are activated by pentobarbital (Parker et al., 1986). Such differences might be due to different subunit isoforms available in total RNA, distinctions in the subunit stoichiometry of expressed receptors, or differences in the extent of chemical modification of subunits.

A striking functional deficit of the receptor expressed from α_1-, β_1-, and γ_2-subunits is the lack of evidence for cooperativity of GABA in gating the channel. The value of the limiting slope of the GABA dose response curve (log-log plot) was 1.06, as compared to a value of nearly 2 for the native receptor (Sakmann et al., 1983) and values of 1.7 and 1.4, respectively, for the receptors expressed from either chick forebrain mRNA (Sigel and Baur, 1988) or rat brain mRNA (Parker et al., 1986). Thus, the structural requirements for GABA cooperativity remain elusive.

In addition to the GABA-gated channels, expression of the α_1-, β_1-, and γ_2-subunits in *Xenopus* oocytes gave rise to a small population of anion channels that are not gated by GABA but are blocked by picrotoxin. This population of channels contributed 0.5% of the I_{max} induced by GABA. GABA-independent channels were observed previously, when the β_1-subunit cRNA alone was expressed in *Xenopus* oocytes (Sigel et al., 1989). Apparently, homomeric β_1-subunit channels exist that are not gated by GABA but are blocked by picrotoxin.

Mapping the sites of subunit gene expression suggests that the α_1-, β_1-, and γ_2-subunits are likely components of $GABA_A$ receptors in some, but not all, brain regions (Table 1). A hybridization signal for all 3 subunits was detected in the mitral cells of the olfactory bulb, pyramidal cells of the hippocampus, granule cells of the dentate gyrus, and cerebellar granule cells. Furthermore, a coexpression is apparent in the cerebral cortex lamina II, III, V, and VI. However, in some brain areas gene expression for only the α_1- and γ_2-subunits, but not the β_1-subunit, was apparent, e.g., in cerebellar Purkinje cells, some thalamic nuclei, colliculus inferior, nucleus interpositus, subbrachial nucleus, and other brain areas outlined in Table 1. Presumably, variants of the β_1-subunit, such as the β_2- or β_3-isoforms, are expressed in these brain regions. In brain areas in which expression of only 1 subunit could be detected (e.g., α_1 in some thalamic nuclei and in the hippocampal stratum oriens), subunits other than β_1 and γ_2 may be identified as further components of the respective $GABA_A$ receptors. Clearly, in the absence of the γ_2-subunit, other yet unknown subunits are expected to provide the structural basis for modulation of the GABA response by BZR ligands.

References

Barish ME (1983) A transient calcium-sensitive chloride current in the immature *Xenopus* oocyte. J Physiol (London) 342:309–325.

Hopfield JF, Tank DW, Greengard P, Huganir RL (1988) Functional modulation of the nicotinic acetylcholine receptor by tyrosine phosphorylation. Nature 336:677–680.

Levitan ES, Schofield PR, Burt DR, Rhee LM, Wisden W, Kohler M, Fujita N, Rodriguez HF, Stephenson A, Darlison MG, Barnard EA, Seeburg PN (1988a) Structural and functional basis for $GABA_A$ receptor heterogeneity. Nature 335:76–79.

Levitan ES, Blair LAC, Dionne VE, Barnard EA (1988b) Biophysical and pharmacological properties of cloned $GABA_A$ receptor subunits expressed in *Xenopus* oocytes. Neuron 1:779–781.

Lolait SJ, O'Carroll AM, Kusano K, Mueller JM, Brownstein MJ (1989) Cloning and expression of a novel rat $GABA_A$ receptor. FEBS Lett 246:145–148.

Malherbe P, Draguhn A, Multhaup G, Beyreuther K, Möhler H (1990) $GABA_A$ receptor expressed from rat brain α_1 and β_1 subunit cDNAs displays potentiation by benzodiazepine receptor ligands. Mol Brain Res 8.

Miledi R, Parker I, Sumikawa K (1982) Synthesis of chick brain GABA receptors by frog oocytes. Proc R Soc Lond (Biol) 216:509–515.

Möhler H, Battersby MK, Richards JG (1980) Benzodiazepine receptor protein identified and visualized in brain tissue by a photoaffinity label. Proc Natl Acad Sci USA 77:1666–1670.

Parker I, Gundersen CB, Miledi R (1986) Actions of pentobarbital on rat brain receptors expressed in *Xenopus* oocytes. J Neurosci 6:2290–2297.

Pritchett DB, Sontheimer H, Gorman CM, Kettenmann H, Seeburg PH, Schofield PR (1988) Transient expression shows ligand gating and allosteric potentiation of $GABA_A$-receptor subunits. Science 242: 1306–1308.

Pritchett, DB, Sontheimer H, Shivers BD, Ymer S, Kettenmann H, Schofield PR, Seeburg PH (1989) Importance of a novel $GABA_A$ receptor subunit for benzodiazepine pharmacology. Nature 338:582–585.

Sakmann B, Hamill OP, Bormann J (1983) Patchclamp measurements of elementary chloride currents activated by the putative inhibitory transmitters GABA and glycine in mammalian spinal cord neurons. J Neural Transmission Suppl 18:83–95.

Sanger F, Coulson A, Barrell B, Smith A, Roe B (1980) Cloning in single-stranded bacteriophage as an aid to rapid DNA sequencing. J Mol Biol 143:161–178.

Schoch P, Häring P, Takacs B, Stähli C, Möhler H (1984) GABA/benzodiazepine receptor from bovine brain: purification, reconstitution and immunological characterization. J Recept Res 4:189–200.

Schofield PR, Darlison MG, Fujita N, Burt DR, Stephenson FA, Rodriguez H, Rhee LM, Ramachandran J, Reale V, Glencorse TA, Seeburg PH, Barnard EA (1987) Sequence and functional expression of the $GABA_A$ receptor shows a ligand-gated receptor super-family. Nature 328:221–227.

Schofield PR, Pritchett DB, Sontheimer H, Kettenmann H, Seeburg PH (1989) Sequence and expression of human $GABA_A$ receptor α_1 and β_1 subunits. FEBS Lett 244:361–364.

Séquier JM, Richards JG, Malherbe P, Price GW, Mathews S, Möhler H (1988) Mapping of brain areas containing RNA homologous to cDNAs encoding the α- and β-subunits of the rat $GABA_A$ receptor. Proc Natl Acad Sci USA 85:7815–7819.

Shivers BD, Killisch I, Sprengel R, Sontheimer H, Köhler M, Schofield PR, Seeburg PH (1989) Two novel $GABA_A$ receptor subunits exist in distinct neuronal subpopulations. Neuron 3:327–337.

Sigel E (1987) Properties of single sodium channels translated by *Xenopus* oocytes after injection with messenger ribonucleic acid. J Physiol (London) 386:73–90.

Sigel E, Barnard EA (1984) A γ-aminobutyric acid/benzodiazepine receptor complex from bovine cerebral cortex: improved purification with preservation of regulatory sites and their interactions. J Biol Chem 259:7219–7223.

Sigel E, Baur R (1988) Allosteric modulation by benzodiazepine receptor ligands of the $GABA_A$ receptor channels expressed in *Xenopus* oocytes. J Neurosci 8:289–295.

Sigel E, Stephenson FA, Mamalaki C, Barnard EA (1983) A γ-aminobutyric acid/benzodiazepine receptor complex of bovine cerebral cortex: purification and partial characterization. J Biol Chem 258: 6965–6971.

Sigel E, Baur R, Malherbe P, Möhler H (1989) The rat β_1-subunit of the $GABA_A$-receptor forms a homomeric, picrotoxin-sensitive anion channel open in the absence of GABA. FEBS Lett 257:377–379.

von Heijne G (1986) A new method for predicting signal sequence cleavage sites. Nucleic Acids Res 14:4683–4690.

Ymer, S, Schofield P, Draguhn A, Werner P, Köhler M, Seeburg PH (1989) $GABA_A$ receptor β-subunit heterogeneity: functional expression of cDNAs. EMBO J 8:1665–1670.