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Role of the β Subunit in Determining the Pharmacology of Human γ-Aminobutyric Acid Type A Receptors

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SUMMARY

A cDNA encoding the human γ-aminobutyric acid $(GABA)_A$ receptor β2 subunit has been cloned and sequenced. The deduced amino acid sequence of this cDNA shows only a single amino acid change from the rat sequence (Asn-347 in rat, serine in human). Using polymerase chain reaction amplification of human-specific products from human × rodent somatic cell hybrid DNAs, the gene has been assigned to human chromosome 6. By expressing recombinant human $GABA_A$ receptors containing different β subunits (β1, β2, or β3) in both transfected cells and *Xenopus* oocytes, we have been able to determine the influence of the β subunit on the pharmacology of the receptor. For a number of benzodiazepine binding site compounds, a barbiturate, and several neurosteroids, neither the affinity nor the efficacy of the compounds is influenced by the type of β subunit present in the receptor molecule. These data suggest that the β subunit does not significantly influence the benzodiazepine, barbiturate, or steroid site pharmacologies of human $GABA_A$ receptor subtypes.

GABA is the major inhibitory neurotransmitter in the mammalian brain. The receptors activated by GABA have been pharmacologically subdivided into $GABA_A$ and $GABA_B$ receptors. The binding of GABA to $GABA_A$ receptors results in the opening of an intrinsic chloride channel and the subsequent hyperpolarization of the cell membrane (1). The function of $GABA_A$ receptors is modulated by a number of drugs, including BZs, barbiturates, ethanol, and neurosteroids (2).

Understanding of the structure of the mammalian $GABA_A$ receptor has recently increased as a result of the application of molecular biological approaches. It is now known that there is a gene family of $GABA_A$ receptor subunits, i.e., six α subunits, three β subunits, three γ subunits, and one δ subunit (3–21). By using *in vitro* expression systems (*Xenopus* oocytes and transfected cells), it has been shown that an α, a β, and a γ subunit are necessary to form receptor molecules that exhibit the properties of native $GABA_A$ receptors (11, 22).

When recombinant receptors assembled from α, β, and γ subunits are expressed, both the α and the γ subunits present have been shown to affect the BZ pharmacology (7, 10, 21–26); however, there has been no definitive study addressing the influence of the β subunit. The markedly different mRNA distributions of the three β subunit isoforms in the mammalian central nervous system (27–29) appear to suggest a functional significance for their existence.

Here we report the cloning and sequencing of a cDNA encoding the human β2 $GABA_A$ receptor subunit, as well as the chromosomal localization of its gene. Additionally, we present the findings of a systematic analysis of the effect of the substitution of different β subunits into otherwise identical subunit combinations on the pharmacologies of a number of ligands at the BZ, neurosteroid, and barbiturate sites on the $GABA_A$ receptor.

Materials and Methods

Cloning of human β2 and β3 $GABA_A$ subunit cDNAs. A human β2 cDNA probe was obtained by PCR from a human cerebellum λZAP II cDNA library (Stratagene), using oligonucleotide primers derived from the intracellular loop of the published rat β2 subunit nucleotide sequence (8) (equivalent positions: sense primer, bp 1230–1261; antisense primer, bp 1473–1507). PCR was performed as described previously (21). A human fetal brain λZAP II cDNA library (Stratagene) was screened with the ^{32}P-labeled β2 cDNA probe as described previously (21), and cDNA clones were sequenced on both strands using Sequenase (United States Biochemicals). A β2 cDNA containing the entire coding region was obtained by assembly of three truncated overlapping β2 cDNAs.

To isolate a human β3 subunit cDNA, a human β3 cDNA probe was first isolated by PCR from pooled human fetal, cerebellar, and hippocampal cDNA libraries (Stratagene), using oligonucleotide primers derived from the published rat β3 sequence (8) (equivalent positions: sense primer, bp 1084–1113; antisense primer, bp 1323–1354). A human fetal brain cDNA library was screened with this ^{32}P-labeled β3 probe,

ABBREVIATIONS: GABA, γ-aminobutyric acid; BZ, benzodiazepine; bp, base pair(s); PCR, polymerase chain reaction; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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and a putative human β3 cDNA clone was obtained. DNA sequencing indicated that this cDNA was human β3, compared with the sequence of a human β3 cDNA published while this work was ongoing (9). The clone was found to contain the entire coding region except the 5′-terminal adenosine nucleotide of the initiation codon ATG. The clone was extended by PCR using a mutagenic 5′ oligonucleotide primer (5′-TGCAGGAATCCATGGGGGGCCTTGCGGGAGGAA-3′) containing the missing adenosine residue and introducing an *Nco*I restriction endonuclease site upstream of the initiation codon ATG, to facilitate subcloning into expression vectors, and a 3′ primer corresponding to the 3′ untranslated region of the human cDNA (9) (antisense primer, bp 1541–1568).

Human chromosomal localization. A set of PCR primers was chosen from DNA sequence obtained from the 3′ untranslated region (30) of the *GABRB2* gene using the primer selection program developed by Lowe *et al.* (31). The sequences of the primers were 5′-TCACTGGTTTAGGATTGGTAGC-3′ and 5′-CTGACAGTATG-TTTGTGC-3′. PCR amplification of the human × rodent somatic cell hybrid mapping panel 1 (Coriell Institute) was done using 50 ng of template DNA in a 25-μl reaction volume containing 1.5 mM $MgCl_2$, 200 μM levels of each deoxynucleoside triphosphate, 200 ng of each primer, and 0.625 units of *Taq* polymerase, in 50 mM KCl, 10 mM Tris·HCl, pH 8.3. Cycling was carried out in a Perkin-Elmer Cetus 9600 thermocycler using a "touchdown" protocol (32), as follows: a 2-min initial denaturation step at 94° and then two cycles of denaturation at 94° for 30 sec, annealing at 54° for 30 sec, and extension at 72° for 30 sec. The program continued in this way, decreasing the annealing temperature by 1° every two cycles until it reached a point 9° below the starting temperature (45°). At this time, another 15 amplification cycles were carried out at the 45° annealing temperature. Products were visualized on a 2.5% 3:1 NuSieve/agarose gel.

Expression vectors and transfections. Isolation of α1, α2, α5, β1, and γ2 human $GABA_A$ receptor subunit cDNAs has been described previously (21). The isoform of the human γ2 subunit used throughout the study was γ2S. α1β1γ2, α2β1γ2, and α5β1γ2 $GABA_A$ receptor subtypes containing β1 or β2 subunits were expressed transiently in human embryonic kidney 293 cells, using standard methods (21, 33). Each subunit cDNA was expressed from vector pCDM8 (Invitrogen).

The subtypes containing the β3 subunit were expressed stably by transfection of the appropriate subunit cDNAs in vector pMSGneo (34, 35) into mouse L(tk$^-$) cells. DNA for transfection was purified by CsCl centrifugation. Cell culture and transfections were performed as described previously (35). Geneticin (GIBCO/BRL)-resistant cell colonies from each of the α1β3γ2, α2β3γ2, and α5β3γ2 stable transfections were isolated with cloning cylinders and individually analyzed for the binding of [^{3}H]Ro15–1788 (83 Ci/mmol; NEN), as described below, after a 7-day induction of receptor expression by the addition of 1 μM dexamethasone to culture medium lacking Geneticin. For each subtype, the population expressing the highest levels of [^{3}H]Ro15–1788 binding was recloned by limiting dilution. The resultant cell lines (H132 clone 16, J232 clone 1, and K532 clone 5) were initially maintained in medium containing Geneticin (2 mg/ml) but were subsequently cultured in normal growth medium and incubated only every 3–4 weeks in medium containing Geneticin.

Membrane preparation and ligand binding. Cells were washed twice with phosphate-buffered saline and scraped into phosphate-buffered saline. After centrifugation (500 × *g*), the cell pellet was resuspended by vortexing in 10 mM potassium phosphate buffer, pH 7.4, and was centrifuged (48,000 × *g* for 30 min at 4°). This procedure was repeated before final resuspension and homogenization in potassium phosphate buffer, pH 7.4, containing 100 mM KCl (assay buffer), using a Semat Ultra-Turrax homogenizer (three 5-sec bursts at setting 5).

Saturation binding curves were obtained by incubating membranes with various concentrations of [^{3}H]Ro15–1788, with nonspecific binding being measured in the presence of 10 μM flunitrazepam. All binding assays were performed for 90 min at 4° in assay buffer. The total assay volume was 0.5 ml, containing approximately 200 μg of membrane protein. Incubations were terminated by filtration through GF/B filters (Brandel, Gathersburg, MD) on a Tomtec cell harvester, followed by three washes in ice-cold assay buffer. After drying, filter-retained radioactivity was determined by liquid scintillation counting. Experimental data points were fitted to single-site dose-response curves using RS/1 software (BBN Research Systems, Cambridge, MA). K_i values were determined from three or more independent experiments, using the equation $K_i = IC_{50}/1 + [[^3H]Ro15–1788]/K_d$. All points on binding curves were derived from triplicate assays. Zolpidem was obtained from Synthelabo and CL218,872 from Lederle; FG8205 was synthesized at Merck Sharp & Dohme (Hoddesdon, Germany). Abecarnil was a gift from Schering AG. All other compounds were obtained from Sigma Biochemicals or Research Biochemicals.

Oocyte expression. *Xenopus* oocytes were removed from anesthetized frogs and manually defolliculated with fine forceps. After mild collagenase treatment (type 1A, 0.5 mg/ml, for 10 min) to remove follicle cells, the oocyte nuclei were directly injected with 10–20 nl of injection buffer (88 mM NaCl, 1 mM KCl, 15 mM HEPES, pH 7.0; nitrocellulose filtered) containing different combinations of human $GABA_A$ subunit cDNAs (6 ng/μl) engineered into the expression vector pCDM8. After incubation for 24 hr, oocytes were placed in a 50-μl bath and perfused with modified Barth's medium consisting of 88 mM NaCl, 1 mM KCl, 10 mM HEPES, 0.82 mM $MgSO_4$, 0.33 mM $Ca(NO_3)_2$, 0.91 mM $CaCl_2$, and 2.4 mM $NaHCO_3$, pH 7.5. Cells were impaled with two 1–3-MΩ electrodes containing 2 M KCl and were voltage-clamped between −40 and −70 mV. The cells were continuously perfused with saline at 6–10 ml/min, and drugs were applied in the perfusate. GABA modulators were preapplied for 30 sec before the addition of GABA. GABA was applied until the peak of the response was observed, usually 30 sec or less. At least 3-min wash time was allowed between each GABA application, to prevent desensitization.

Results

The nucleotide and deduced amino acid sequences of the human β2 $GABA_A$ receptor subunit cDNA are shown in Fig. 1. The coding region of 474 amino acids differs from the rat sequence only at position 347 (serine in the human sequence and asparagine in the rat sequence) and contains the expected motifs common to the ligand-gated ion channel superfamily, i.e., a putative amino-terminal signal peptide, a pair of cysteines separated by 13 residues, four putative membrane-spanning domains with a large intracellular loop between transmembrane domains 3 and 4, and a large putative extracellular domain. The amino acid sequence shows 78% identity with the human β1 subunit sequence (19) and 81% identity with the human β3 subunit sequence (9). Alignment of the three human β subunit sequences (Fig. 2) shows that the majority of amino acid differences are found in the putative signal peptide and the putative large cytoplasmic loop.

The human chromosomal localization of the β2 subunit gene was performed using PCR amplification of human-specific β2 gene sequences from a human × rodent somatic cell hybrid mapping panel. The primers amplified a 105-bp product from human and hamster but not mouse genomic DNA. All hybrids in which >10% of cells contained human chromosome 6 generated this product. The only discordant hybrid, hybrid 18, was not included in this analysis because it was a hamster × human hybrid and hamster genomic DNA generated the same product as did human DNA. (All other hybrids in this panel were derived from the mouse.) Excluding hybrid 18, the concordance frequency for the *GABRB2* gene was 100% for chromosome-containing cell hybrids, whereas the next highest concordance was 88% for chromosome 4 (15 of 17 cell hybrids).

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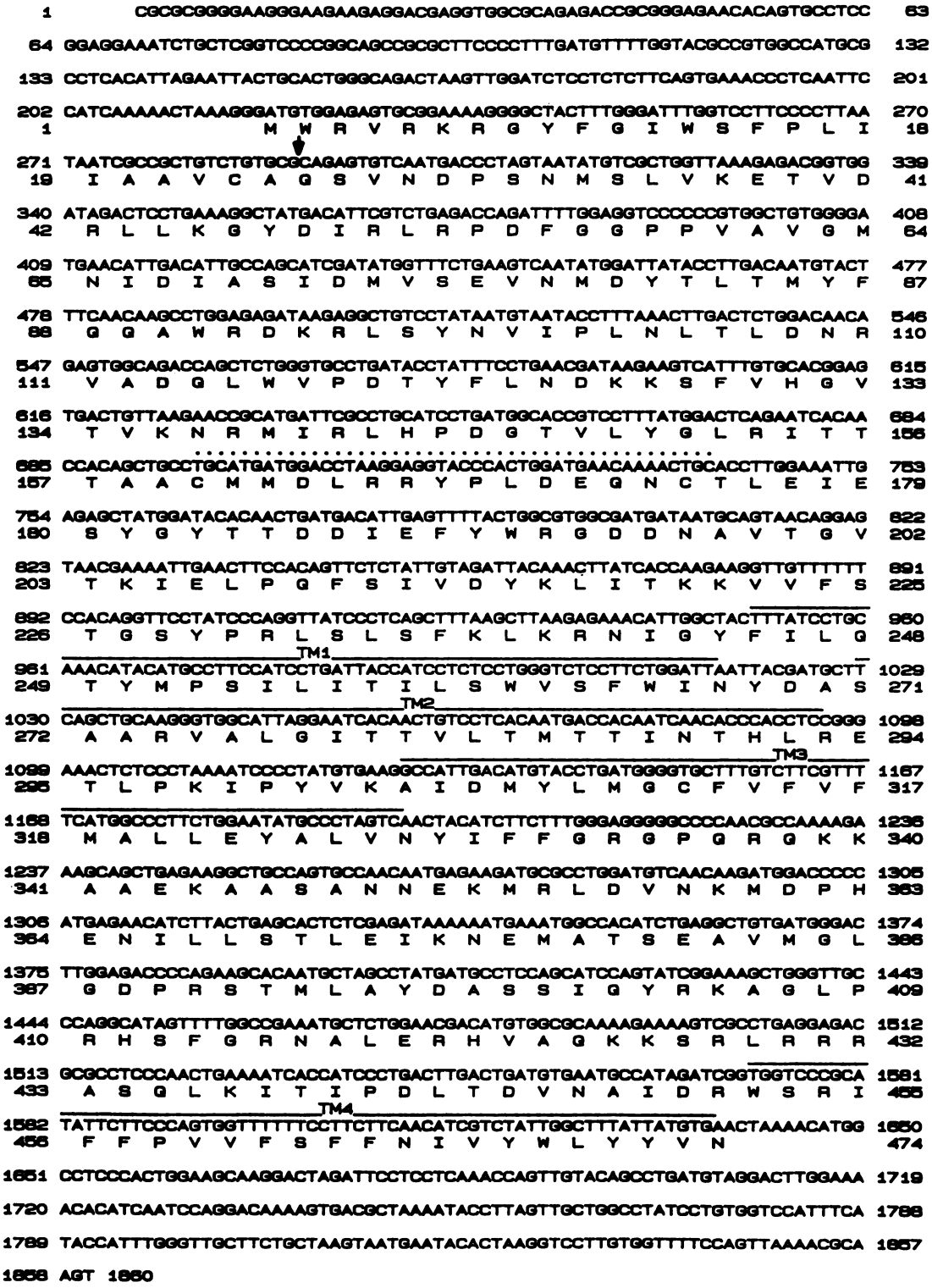

Fig. 1. Nucleotide and predicted amino acid sequences of the human GABA$_A$ receptor $\beta 2$ subunit. *Arrow*, position of the putative signal sequence cleavage site. The four transmembrane domains (*TM1-TM4*) are *overlined* and the two conserved cysteine residues separated by 13 amino acids are indicated by a *dotted line*.

Stable cell lines expressing $\alpha 1\beta 3\gamma 2S$, $\alpha 2\beta 3\gamma 2S$, or $\alpha 5\beta 3\gamma 2S$ GABA$_A$ receptor subtypes were produced by transfection of mouse L(tk$^-$) cells with individual subunit cDNAs in the vector pMSGneo. We previously reported this approach for the production of a bovine $\alpha 1\beta 1\gamma 2L$-expressing stable cell line (35). Geneticin-resistant transfectants were screened for binding of [^{3}H]Ro15–1788, and the majority ($\alpha 1\beta 3\gamma 2$, 8 of 11 cell hybrids; $\alpha 2\beta 3\gamma 2$, 37 of 44 cell hybrids; $\alpha 5\beta 3\gamma 2$, 21 of 27 cell hybrids) specifically bound >40 fmol of radioligand/10-cm plate of cells. The populations exhibiting the highest levels of binding were then recloned by limiting dilution, to produce cell lines H132 clone 26, J232 clone 1, and K532 clone 5, respectively. Each

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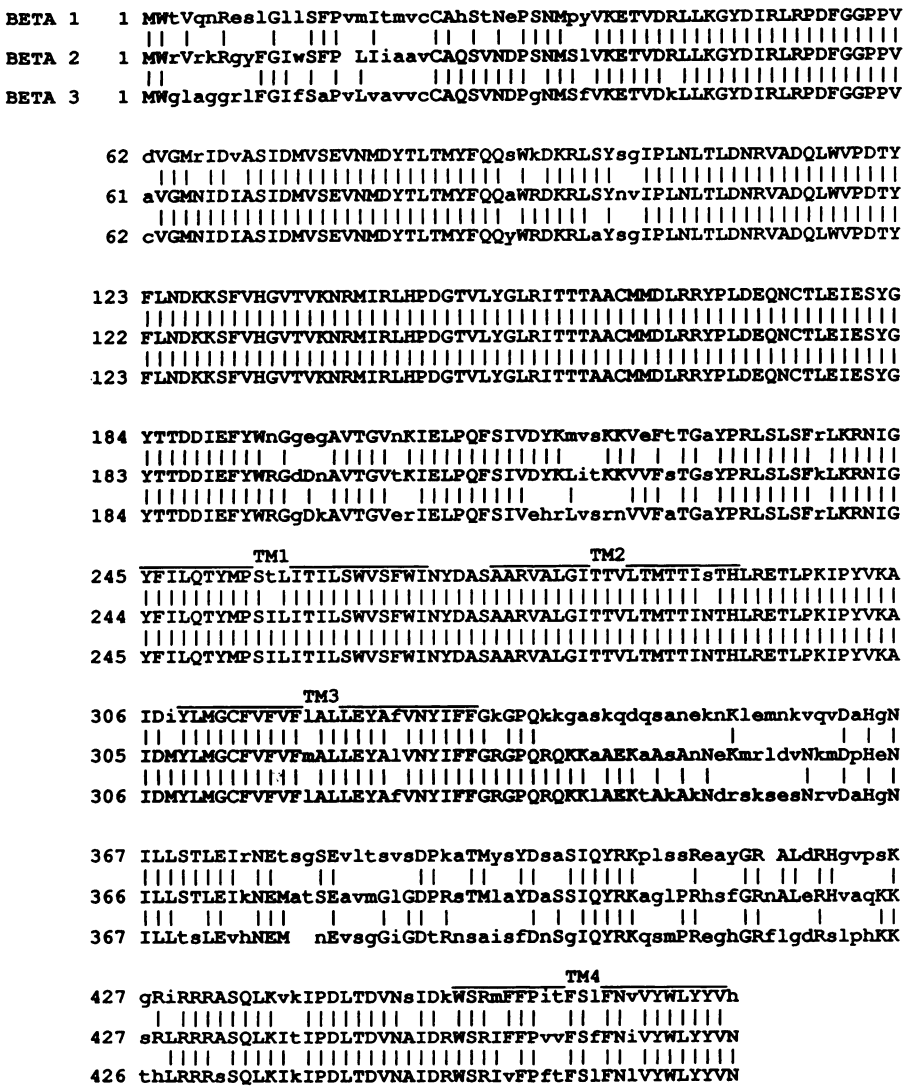

Fig. 2. Alignment of the deduced amino acid sequences of the human $GABA_A$ receptor $\beta1$ (19), $\beta2$, and $\beta3$ (9) subunits. Sequences were aligned using the Genalign program (Intelligenetics, Palo Alto, CA) so that the most homologous sequences are placed next to one another. The amino acid numbers are indicated on the *left*. *TM1-TM4*, putative transmembrane domains.

cell line expressed >0.5 pmol of [^{3}H]Ro15–1788 binding sites/mg of protein after 7 days of induction in medium containing dexamethasone. Similarly, transient expression of $\alpha1\beta1\gamma2$, $\alpha1\beta2\gamma2$, $\alpha2\beta1\gamma2$, $\alpha2\beta2\gamma2$, $\alpha5\beta1\gamma2$, and $\alpha5\beta2\gamma2$ $GABA_A$ receptor subtypes in human embryonic kidney 293 cells yielded between 0.1 and 1.0 pmol of [^{3}H]Ro15–1788 binding sites/mg of membrane protein.

Scatchard analysis of saturation binding curves for [^{3}H]Ro15–1788 were performed for each of the nine receptor subtypes studied, giving the K_d values shown in Table 1. Displacement of sub-K_d concentrations of this radioligand was used to generate competitive binding curves for a number of BZ site ligands, including BZ-1-selective (zolpidem) and nonselective (flunitrazepam and triazolam) full agonists, both nonselective (bretazenil and FG8205) and BZ-1-selective (CL218,872) partial agonists, a partial inverse agonist (Ro15–4513) previously shown to be $\alpha5$ selective (21), and a full inverse agonist (methyl-β-carboline-3-carboxylate). The K_i values obtained from these assays are also presented in Table 1. There was no appreciable difference in the ligand binding affinities for any BZ site ligand studied between subtypes differing only in the β subunit present. The greatest variation seen between K_i values in such subtype comparisons was with zolpidem, which with $\alpha1$ and $\alpha2$ subtypes gave mean values 2–3-fold higher for $\beta1$- than for $\beta2$-containing combinations, with $\beta3$-containing receptors giving intermediate values. The lack of effect of the β subunit on the BZ pharmacology is emphasized in Fig. 3, where the data from Table 1 are expressed as correlation plots. In each case, linear regression analysis using RS/1 software was used and a correlation coefficient close to unity was obtained ($\alpha1/2/5\beta1\gamma2$ versus $\alpha1/2/5\beta2\gamma2$, $r^2 = 0.98$; $\alpha1/2/5\beta1\gamma2$ versus $\alpha1/2/5\beta3\gamma2$, $r^2 = 0.97$; $\alpha1/2/5\beta2\gamma2$ versus $\alpha1/2/5\beta3\gamma2$, $r^2 = 0.98$).

To address the functional consequences of $GABA_A$ receptors containing different β subunits, three $GABA_A$ receptor subunit combinations were expressed in *Xenopus* oocytes, i.e., $\alpha1\beta1\gamma2$, $\alpha1\beta2\gamma2$, and $\alpha1\beta3\gamma2$. The pharmacological properties of these receptors were then compared using two-electrode voltage-clamp electrophysiological recording. The GABA affinities for these different combinations were 25 ± 4.1 μM ($n = 8$), 20 ± 2.8 μM ($n = 9$), and 8 ± 2 μM ($n = 5$) (mean ± standard error), respectively, with $\beta3$-containing receptors thus having a significantly higher affinity than either $\beta1$-containing ($p \leq 0.005$) or $\beta2$-containing ($p \leq 0.01$) receptors. An EC_{20} concentration of GABA for each individual oocyte was used to study the effects of different modulators on the function of the $GABA_A$ receptor. The modulation of all three receptors by a number of BZs, the

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TABLE 1

Affinities of selected BZ binding site ligands for various human GABA$_A$ receptor subunit combinations expressed in transfected cells

Affinities (K_i; K_d where indicated) for nine BZ site ligands are shown. K_d values were obtained by Scatchard isotherm analysis of radioligand binding. The K_i values shown were determined by displacement of sub-K_d quantities of [^{3}H]Ro15-1788 by the various ligands, as described in detail in Materials and Methods. Values given are mean ± standard error of at least three independent determinations. The values for $\alpha1\beta1\gamma2$, $\alpha2\beta1\gamma2$, and $\alpha5\beta1\gamma2$ have been taken from the report of Hadingham *et al.* (21).

Ligand	K_i								
	$\alpha1\beta1\gamma2$	$\alpha1\beta2\gamma2$	$\alpha1\beta3\gamma2$	$\alpha2\beta1\gamma2$	$\alpha2\beta2\gamma2$	$\alpha2\beta3\gamma2$	$\alpha5\beta1\gamma2$	$\alpha5\beta2\gamma2$	$\alpha5\beta3\gamma2$
					nM				
[^{3}H]Ro15-1788[a]	0.81 ± 0.07	1.01 ± 0.06	0.92 ± 0.04	0.90 ± 0.09	1.12 ± 0.04	1.05 ± 0.05	0.56 ± 0.03	0.37 ± 0.01	0.45 ± 0.04
Flunitrazepam	11.45 ± 1.57	7.96 ± 0.15	22.44 ± 2.47	5.17 ± 0.44	3.34 ± 0.16	9.57 ± 1.59	5.48 ± 0.40	7.02 ± 0.29	12.08 ± 1.33
β-CCM[b]	2.15 ± 0.42	1.74 ± 0.12	3.78 ± 1.08	6.45 ± 1.19	6.50 ± 0.55	15.69 ± 1.09	76.41 ± 7.76	125.4 ± 1.84	260.6 ± 50.84
Ro15-4513	10.03 ± 0.62	10.36 ± 0.46	8.85 ± 0.90	10.37 ± 1.10	5.46 ± 0.12	8.10 ± 2.46	0.69 ± 0.14	0.48 ± 0.04	0.99 ± 0.23
FG8205	2.33 ± 0.54	1.77 ± 0.07	2.29 ± 0.52	3.65 ± 0.15	3.75 ± 0.21	6.62 ± 1.88	6.35 ± 0.03	3.66 ± 0.13	5.89 ± 1.18
CL218,872	290.5 ± 31.88	220.3 ± 37.47	301.3 ± 28.39	2,903 ± 420.2	1,058 ± 211.1	3,470 ± 903.3	1,154 ± 66.23	2,624 ± 200.5	1,835 ± 803.4
Zolpidem	111.9 ± 16.96	59.63 ± 20.08	64.15 ± 7.52	760.6 ± 88.29	291.4 ± 10.12	427.0 ± 31.22	>15,000	>15,000	>15,000
Triazolam	1.84 ± 0.44	ND[c]	3.00 ± 0.85	1.20 ± 0.19	ND	1.87 ± 0.29	1.23 ± 0.27	ND	2.74 ± 0.24
Bretazenil	1.17 ± 0.35	ND	1.17 ± 0.35	1.20 ± 0.23	ND	2.07 ± 0.24	2.44 ± 0.48	ND	2.11 ± 0.47

[a] K_d values.
[b] β-CCM, methyl-β-carboline-3-carboxylate.
[c] ND, not determined.

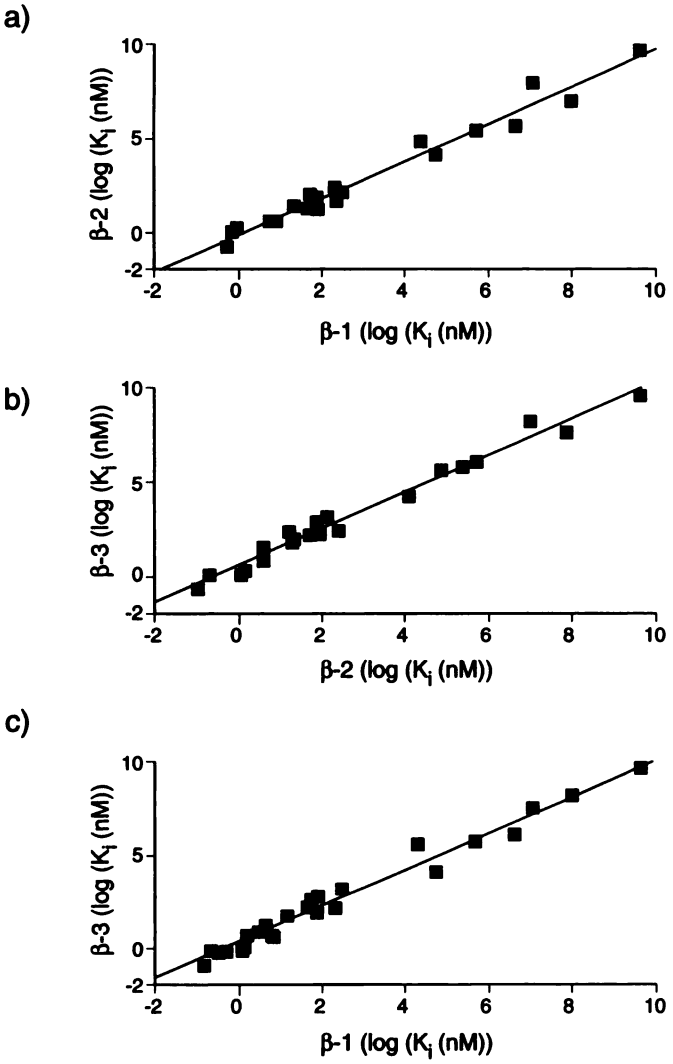

Fig. 3. Correlation between the affinities of BZ site ligands for human GABA$_A$ receptor subtypes containing different β subunits. a, $\alpha1/2/5\beta2\gamma2$ versus $\alpha1/2/5\beta1\gamma2$; b, $\alpha1/2/5\beta3\gamma2$ versus $\alpha1/2/5\beta2\gamma2$; c, $\alpha1/2/5\beta3\gamma2$ versus $\alpha1/2/5\beta1\gamma2$. Data are taken from Table 1.

barbiturate pentobarbital, and three different steroids was investigated. The potentiation by the BZ full agonists flunitrazepam and zolpidem and the partial agonists FG8205 and CL218,872 and the inhibition by the inverse agonist methyl-4-ethyl-6,7-dimethoxy-β-carboline-3-carboxylate were similar for all three β-containing receptors, demonstrating that BZ efficacy was not influenced by the β subunit variant (Fig. 4). This was also true for compounds acting at the neurosteroid modulatory site (Fig. 5); 5β-pregnan-3α-ol-20-one (pregnanolone) and 5α-pregnan-3α-ol-20-one both potentiated the GABA response to the same level with all three receptor subtypes. A third steroid, dehydroepiandrosterone, which is an inverse agonist at the steroid site, also inhibited responses to the same degree with $\alpha1\beta1\gamma2$, $\alpha1\beta2\gamma2$, and $\alpha1\beta3\gamma2$ receptors. Finally, the barbiturate pentobarbital also displayed no significant difference in its level of potentiation with receptors containing different β subunits (Fig. 5).

Discussion

We report here the cloning and sequencing of a cDNA encoding the human β2 GABA$_A$ receptor subunit. Additionally, by expressing in *Xenopus* oocytes or transfected cells human GABA$_A$ receptors containing β1, β2, or β3 subunits, we have investigated the influence of the β subunit on the pharmacology of GABA$_A$ receptor subtypes.

Cloning and sequencing of a cDNA encoding a human β2 subunit generated sequence of the 3′ untranslated region of the gene, which could be used for assignment of the human β2 gene to chromosome 6. It is interesting to note that the genes for the other β subunits, β1 and β3, have been assigned to chromosomes 4 and 15, respectively (36, 37). Thus, although it is likely that these genes arose by duplication from an 'ancestral' β subunit, they have not remained linked on the same chromosome but rather have been dispersed throughout the genome. This is also true for the genes of the α and γ subunit classes that to date have been assigned to a chromosome (36, 38, 39). The chromosomal assignment of a gene can be the initial step in determining the link between that gene and an inherited disorder. It has been speculated that members of the GABA$_A$ receptor gene family are candidate genes for epilepsy (40). In this context, we have noted that idiopathic generalized epilepsy, which constitutes 7–8% of all epilepsies, has been localized to

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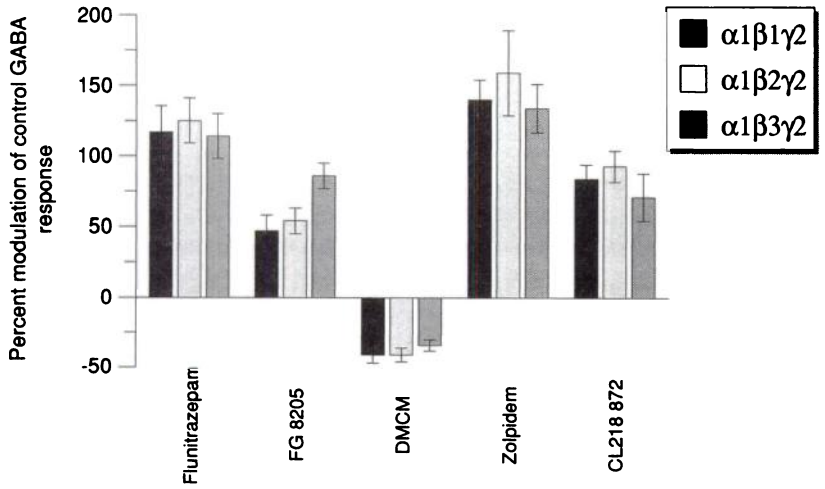

Fig. 4. Comparison of the percentage modulation of the control GABA response by a number of BZ site ligands at $\alpha1\beta1\gamma2$, $\alpha1\beta2\gamma2$, and $\alpha1\beta3\gamma2$ human $GABA_A$ receptors expressed in *Xenopus* oocytes. Each value is the mean ± standard error of at least four oocytes. Control GABA responses were obtained by selecting a GABA concentration approximately 20% of maximum for each individual oocyte. All drugs except methyl-4-ethyl-6,7-dimethoxy-β-carboline-3-carboxylate (*DMCM*) (300 nM) were applied at a concentration of 1 μM.

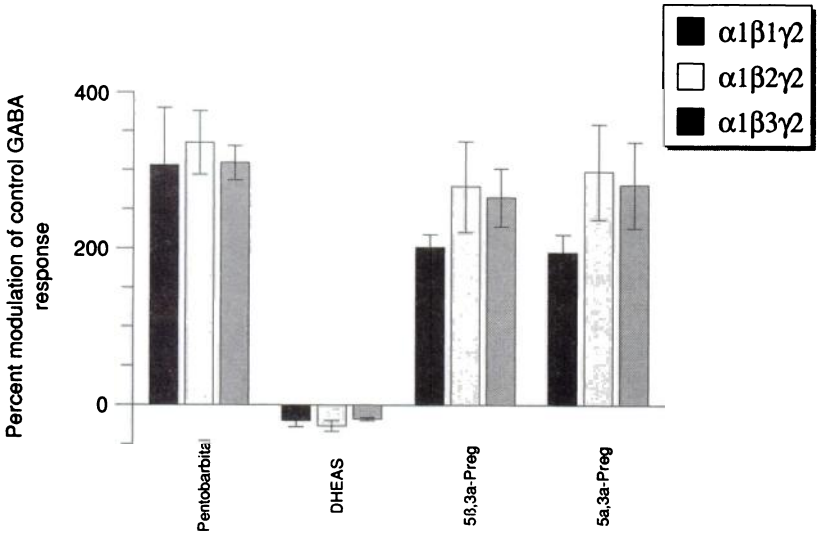

Fig. 5. Comparison of the percentage modulation of the control GABA response by a number of neurosteroid site ligands and a barbiturate at $\alpha1\beta1\gamma2$, $\alpha1\beta2\gamma2$, and $\alpha1\beta3\gamma2$ human $GABA_A$ receptors expressed in *Xenopus* oocytes. Each value is the mean ± standard error of at least four oocytes. All drugs were applied at a concentration of 1 μM. *DHEAS*, dihydroepiandrosterone; *5β,3a-Preg*, 5β-pregnan-3α-ol-20-one; *5a,3a-Preg*, 5α-pregnan-3α-ol-20-one.

chromosome 6p (41). Additional studies are underway to determine whether $\beta2$ is indeed involved in this form of epilepsy.

Previous studies on the pharmacology of recombinant $GABA_A$ receptors have focused on the role of the α and γ subunits. In this study, we have attempted to address the role of the β subunit. It is clear from the data in Table 1 and Figs. 3 and 4 that, for the compounds tested, the type of β subunit present in the receptor does not significantly influence the affinity or efficacy of compounds acting at the BZ binding site. This is in agreement with the findings of Pritchett *et al.* (22), who reported that bovine and rat recombinant receptors containing $\beta1$, $\beta2$, or $\beta3$ had the same affinities for CL218,872 and for Ro15–1788. However, Sigel *et al.* (24) reported several differences between receptors containing $\beta1$ and $\beta2$ subunits. Those authors observed a large difference in GABA affinity between $\alpha1\beta1\gamma2$ and $\alpha1\beta2\gamma2$ (EC_{50} values of 74 μM and 1 mM, respectively) but no such difference between $\alpha5\beta1\gamma2$ and $\alpha5\beta2\gamma2$ (EC_{50} values of 17 μM and 14 μM, respectively). The low GABA affinities for $\alpha1$-containing combinations were later attributed to a mutation in that subunit (42). Additionally, those authors found that BZ potentiation of the GABA response was observed only with combinations containing $\beta2$, and not $\beta1$, subunits. Other studies (22, 25, 26, 43, 44), as well as the original description of the $\gamma2$ subunit (11), agree with the findings reported here showing that receptors containing $\beta1$ are modulated by BZs.

In contrast to the lack of influence of the β subunit, there is a body of evidence demonstrating that both the α subunit (21, 22, 25, 43, 45, 46) and the γ subunit (10, 23, 26, 43) determine both the affinity and the efficacy of compounds acting at the BZ binding site. These data clearly suggest that the BZ modulatory site is formed from determinants from both the α subunit and the γ subunit, which is in accord with the photoaffinity labeling experiments of Stephenson *et al.* (47).

The nature of both the barbiturate and the neurosteroid modulatory sites of the $GABA_A$ receptor has been less well defined than that of the BZ binding site. Here we have presented evidence that the type of β subunit present in the receptor complex does not affect the pharmacology of these two modulatory sites. Previous studies have shown that channels formed by the $\beta1$ subunit alone can be modulated by steroids (48) and that the type of α subunit present influences the sensitivity of recombinant receptors to steroids (49). However, the physical location of the steroid modulatory site(s) on the $GABA_A$ receptor remains unknown.

In this study, we have examined the influence of the β subunit on the pharmacology of the $GABA_A$ receptor. With the exception of an approximately 3-fold higher affinity of GABA for

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β3-containing receptors, compared with β2- or β1-containing receptors, we have not observed any significant influence of the β subunit. Within the rat brain, there is clearly a distinct temporal and regional pattern of expression for each β subunit (27–29), suggesting an important functional role. Additional extensive studies will be required to determine the nature of this role.

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