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Stable expression of mammalian type A γ-aminobutyric acid receptors in mouse cells: Demonstration of functional assembly of benzodiazepine-responsive sites

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ABSTRACT **The differential sensitivity of type A γ-aminobutyric acid ($GABA_A$) receptors to benzodiazepine ligands seen in the mammalian nervous system is thought to be generated by the existence of a number of different receptor subtypes, assembled from a range of closely related subunits (α_{1-6}, β_{1-3}, γ_{1-3}, and δ) encoded by discrete genes. The characteristics of a given subtype can be determined by the coexpression of cloned cDNAs encoding the subunits of interest. Two transient expression systems have so far been employed in the study of the ligand-binding characteristics and chloride channel properties of such $GABA_A$ receptors—*Xenopus* oocytes and transfected mammalian cells. Here we report on the use of a steroid-inducible promoter expression system for the production of a permanently transfected clonal cell line expressing the $\alpha_1\beta_1\gamma_{2L}$ $GABA_A$ receptor subtype. Using both immunoprecipitation by subunit-specific antisera and gel-exclusion chromatography, we have shown that the α_1, β_1, and γ_{2L} subunits coassemble to form receptor macromolecules that are of the same size as native $GABA_A$ receptors. Additionally, the recombinant receptors have the same benzodiazepine pharmacology as native α_1-containing $GABA_A$ receptors and function as GABA-gated chloride channels. Such cell lines expressing individual $GABA_A$ receptor subtypes will prove important tools in the study of the structure, function, and pharmacology of $GABA_A$ receptors and in the development of subtype-specific drugs.**

In the mammalian brain, type A γ-aminobutyric acid ($GABA_A$) receptors are the major mediators of inhibitory neurotransmission. They constitute GABA-gated chloride channels, whose activity may be allosterically modulated by a number of drugs, including the barbiturates and benzodiazepines (BZs) (1). Heterogeneity in the responses of the mammalian receptors to BZ-type ligands has been attributed to the differential assembly of $GABA_A$ receptor subtypes from a family of subunits (α_{1-6}, β_{1-3}, γ_{1-3}, and δ) that have been identified by recombinant DNA approaches (2–14).

As individual $GABA_A$ receptor subtypes are not known to be expressed in any transformed cell lines, it has proved necessary to employ other means for their *in vitro* analysis. Transient expression of various subunit combinations in *Xenopus* oocytes (2–7) and human embryonic kidney 293 cells (7–11, 15–17) have been used to investigate their functional and ligand-binding properties. These approaches have suggested that, while there is a requirement for an α subunit, a β subunit, and the γ_2 subunit in order to allow BZ binding (8), the relative affinities for various ligands, as classically defined by BZ-1 or BZ-2 pharmacology (18), correlate with the type of α subunit present (8, 10, 16). However, both these transient expression approaches are, for many purposes, less convenient and reproducible than a permanent expression system such as a stably transfected cell line. Here we report the production and analysis of a clonal cell line in which high levels of a single $GABA_A$ receptor subtype are expressed. The recombinant receptors have the structural, pharmacological, and functional properties expected of a native $GABA_A$ receptor.

MATERIALS AND METHODS

Expression Vectors and Transfections. The mouse mammary tumor virus (MMTV) promoter-containing vector pMSGneo (19) was used for expression of the cDNAs. The vector was modified slightly by the removal of internal *Hin*dIII sites and the addition of a *Hin*dIII site in the polylinker. The entire bovine α_1 and β_1 subunit cDNAs (2) were then subcloned into the modified pMSGneo. The bovine γ_{2L} subunit cDNA (12) was modified for transfection by addition of 5′ untranslated-region sequence derived from the bovine α_1 clone. Briefly, oligonucleotides 5′-GCGGAGCGAGGATCCCCCTCCGCT-3′ and 5′-CTTCATTGCAAGCTTGTGCAGCTCG-3′, containing a *Bam*HI and *Hin*dIII site, respectively, were synthesized and used in a polymerase chain reaction (PCR) as previously described (12) to obtain a DNA fragment corresponding to bases 49–211 of the bovine α_1 5′ untranslated region. This PCR product was then digested with *Bam*HI and *Hin*dIII and subcloned into *Hin*dIII (partial digestion)/*Bam*HI-digested γ_{2L} cDNA, yielding $\gamma_{2.10L}$, which was then subcloned into pMSGneo.

For transfection, DNA was purified by CsCl centrifugation (20). Cell culture and transfections of mouse L(tk$^-$) cells were performed as described (19). Geneticin (GIBCO/BRL)-resistant cell colonies were isolated with cloning cylinders and individually analyzed for binding of [^{3}H]Ro15-1788 (NEN; 83 Ci/mmol; 1 Ci = 37 GBq), as described below. The cell clone expressing the highest levels of [^{3}H]Ro15-1788 binding (1D1) was subsequently recloned by limiting dilution. The resulting cell line (1D1-PA3) was initially maintained in medium containing Geneticin (2 mg/ml) but was subsequently cultured in normal growth medium and incubated only every 3–4 weeks in medium containing Geneticin.

RNA Analysis. $GABA_A$ receptor subunit RNA expressed by transfected cells was analyzed (19) 5 days after induction of expression by the inclusion of 1 μM dexamethasone in the culture medium. Hybridization of Northern blots was performed with randomly primed ^{32}P-labeled probes prepared from the inserts of bovine α_1, β_1, and γ_{2L} cDNAs. Hybrid-

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Abbreviations: GABA, γ-aminobutyric acid; BZ, benzodiazepine; MMTV, mouse mammary tumor virus.

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ization was performed at high stringency in 5× SSPE (1× SSPE is 0.18 M NaCl/10 mM sodium phosphate, pH 7.4/1 mM EDTA)/50% formamide at 42°C. Filters were washed at 65°C in 0.3× SSPE and exposed to Kodak XAR film for 6 hr at −70°C with a Cronex QIII intensifying screen.

Membrane Preparation and Ligand Binding. Five to 10 days after dexamethasone induction of expression, cells were scraped into Tris-buffered saline (TBS: 137 mM NaCl/3 mM KCl/25 mM Tris·HCl, pH 7.4) and centrifuged at 100 × *g* for 10 min. The cell pellet was homogenized in 50 mM Tris·HCl pH 7.4 containing proteinase inhibitors [phenylmethylsulfonyl fluoride, 1 mM; aprotinin (Sigma), 0.1% (vol/vol); benzamidine hydrochloride, 1 mM; leupeptin, 10 μg/ml; iodoacetamide, 1 mM; bacitracin, 200 μg/ml; soybean trypsin inhibitor, 20 μg/ml in a Semat Ultra-Turrax homogenizer (three 5-sec bursts at setting 5) and then centrifuged at 48,000 × *g* for 30 min at 4°C. The pellets were washed twice in the same buffer by resuspension and centrifugation, then resuspended in 5 mM Tris·HCl, pH 7.4/1 mM EDTA (assay buffer) with proteinase inhibitors at 4°C.

Saturation binding curves were obtained by incubating membrane with various concentrations of [^{3}H]Ro15-1788, with nonspecific binding measured in the presence of 10 μM flunitrazepam. All binding assays were performed for 1 hr at 4°C in assay buffer. The total assay volume was 0.5 ml, containing ≈200 μg of membrane protein. Incubations were terminated by filtration through GF/B filters (Brandel, Gaithersburg, MD) followed by three 3-ml washes with ice-cold assay buffer. Experimental data points were fitted to single-site dose–response curves by using RS/1 software (BBN Research Systems, Cambridge, MA). K_i values were determined from three or more independent experiments. All points on binding curves were derived from triplicate assays.

Western Blot Analysis. Induced 1D1-PA3 and untransfected L(tk$^-$) cell membranes in assay buffer with proteinase inhibitors were subjected to SDS/9% PAGE (40 μg of protein per well), the proteins were transferred to nitrocellulose (Schleicher & Schüll), and unbound sites were blocked by overnight incubation in TBS with 5% milk powder, as described (21). Western blots were probed with a 1:500 dilution of subunit-specific antiserum to the α_1 subunit (21) or β and γ subunits (rabbit polyclonal antisera raised to Met^{331}–His^{396} of bovine β_1 and Leu^{317}–Met^{404} of bovine γ_{2L} subunits, respectively, will be described in detail elsewhere), as described (21). Bands were visualized by using horseradish peroxidase-linked second antibody and TM Blue (Transgenic Sciences, Milford, MA).

Gel Exclusion Chromatography and Immunoprecipitation of Solubilized $GABA_A$ Receptors. Receptors were solubilized from 1D1-PA3 cells and rat brain essentially as described (22). Membranes (2–5 mg of protein per ml) in 10 mM Tris·HCl, pH 7.5/1 mM EDTA/10% (vol/vol) glycerol/1 M NaCl with proteinase inhibitors at 4°C were solubilized in 0.7% (wt/vol) sodium deoxycholate, homogenized, and centrifuged to remove particulate material. Gel exclusion chromatography was carried out (22) in 100 mM Tris·HCl pH 7.5/1 mM EDTA/10% (vol/vol) glycerol/0.2% (vol/vol) Lubrol/0.5 M NaCl at room temperature on a Superose 6 column (1 cm × 31 cm) with a Pharmacia FPLC system. Marker proteins used for calibration were thyroglobin (669 kDa), ferritin (440 kDa), catalase (232 kDa), and aldolase (158 kDa). Blue dextran 2000 was used to measure the void volume, and 100 μl volumes of the fractions were assayed for [^{3}H]muscimol (NEN; 17.1 Ci/mmol) and [^{3}H]Ro15-1788 binding to determine the point of elution of receptor.

For immunoprecipitation, both crude solubilized transfected cell membrane preparations and the pooled FPLC column fractions containing eluted receptor were incubated with concentrations of each of the anti-subunit antibodies previously found to maximally immunoprecipitate receptor, immobilized on protein A-Sepharose (Sigma) as described (21), and the percentage of total [^{3}H]Ro15-1788 binding immunoprecipitated by the antibody was determined.

Electrophysiology. Cells plated on poly(L-lysine)-treated coverslips were superfused with 124 mM NaCl/5 mM KCl/2 mM $CaCl_2$/1 mM $MgCl_2$/5 mM Hepes/11 mM D-glucose (adjusted to pH 7.2 with NaOH). Cells were then patch-clamped in the whole-cell configuration at −60 mV by using pipettes filled with 130 mM CsCl/1 mM $MgCl_2$/10 mM Hepes/11 mM EGTA (to pH 7.3 with CsOH and HCl). Series resistance compensation was not employed, because the data reported here is largely qualitative in nature. Drugs were applied rapidly via a double-barreled pipette (23). To investigate the effects of modulators of $GABA_A$ responses, GABA (3 μM; 2–3 sec) was applied at intervals of 30 sec, and stable currents were obtained. Modulators were then added, and the effects produced were assessed as percent potentiations. Values are expressed as mean ± SEM.

RESULTS

Production of a Cell Line Expressing $\alpha_1\beta_1\gamma_{2L}$ $GABA_A$ Receptors. Mouse L(tk$^-$) cells were transfected with bovine α_1, β_1, and γ_{2L} $GABA_A$ receptor subunit cDNAs subcloned into the eukaryotic expression vector pMSGneo (19). This vector has the dexamethasone-inducible MMTV promoter controlling the expression of cDNA sequence. Without addition of dexamethasone to the transfected cell culture medium there should be little or no expression. This inducible expression system was used because it was postulated that constitutive expression in a transfected cell of a large number of exogenous chloride channels, which may be spontaneously opening, would be toxic.

Geneticin-resistant transfectants were screened for binding of the BZ antagonist [^{3}H]Ro15-1788. Of 66 colony-derived populations, 17 bound in excess of 25 fmol of [^{3}H]Ro15-1788 per 10-cm plate of cells. The population exhibiting the most binding (1D1) was then recloned by limiting dilution, to produce cell line 1D1-PA3. Expression of [^{3}H]Ro15-1788 binding sites after addition of dexamethasone to cell cultures was rapid, plateauing after about 5 days (data not shown) at ≈20,000 [^{3}H]Ro15-1788 binding sites per cell and 650 fmol of [^{3}H]Ro15-1788 bound per mg of protein. This cell line has been in continuous passage for >120 cell doublings with no detectable loss of $GABA_A$ receptor expression, and cell stocks have been frozen and recovered up to three times with no significant change in expression levels.

The expression of the recombinant $GABA_A$ receptors was confirmed by Northern blot analysis (Fig. 1*A*). Single bands corresponding to the β_1 and γ_{2L} subunit mRNAs were visualized. For the α_1 subunit, two major mRNA species were detected. This may represent use of both the endogenous polyadenylylation signal present in the cDNA and the vector-derived polyadenylylation sequence or differing degrees of polyadenylylation. No subunit mRNA could be detected in the absence of dexamethasone induction, confirming the stringency of the MMTV promoter under the cell culture conditions used.

Subunits of the Recombinant Receptor Are of the Expected Sizes. The molecular sizes of the recombinant receptor subunits were determined by Western blot analysis using subunit-specific antisera. Inclusion of identically treated untransfected L(tk$^-$) cell membranes in adjacent lanes acted as a control for nonspecific immunoreactivity. Specific bands corresponding to the α_1 and β_1 subunits could be visualized in 1D1-PA3, but not in control, cell lanes (Fig. 1*B*). The anti-α_1 antibody revealed a polypeptide of 51 kDa, in excellent agreement with previously determined values for the native α_1 subunit (21, 24, 25). The anti-β subunit antibody revealed proteins of 50 kDa and 52 kDa, again in general agreement with the size reported for native β_2 and β_3 subunits (26). The smaller protein may be a proteolytically nicked

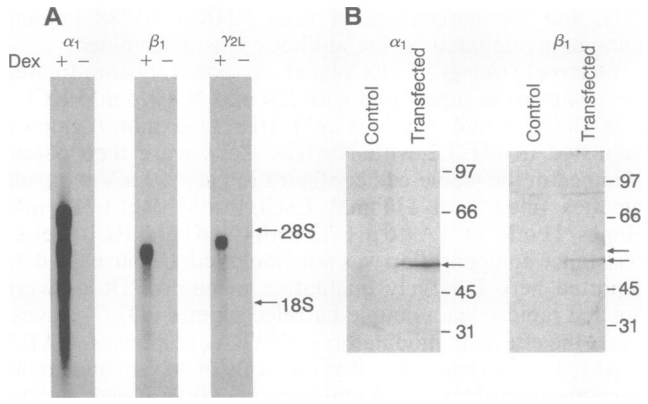

FIG. 1. (*A*) Northern blot analysis of RNA from 1D1-PA3, a stably transfected cell line expressing $\alpha_1\beta_1\gamma_{2L}$ $GABA_A$ receptors. RNA was extracted from cells with (+) or without (−) induction by dexamethasone (Dex). Blots were hybridized under high-stringency conditions with randomly primed ^{32}P-labeled probes prepared from bovine α_1, β_1 (2), and γ_{2L} (12) cDNAs. Positions of 28S and 18S rRNA are indicated. (*B*) Western blot analysis of membranes from control L(tk$^-$) cells and 1D1-PA3 transfectants. Cell membranes were subjected to SDS/9% PAGE, and the separated proteins were blotted onto nitrocellulose and probed with anti-α_1 (*Left*) and -β_1 (*Right*) antibodies. Molecular size markers (kDa) are at right.

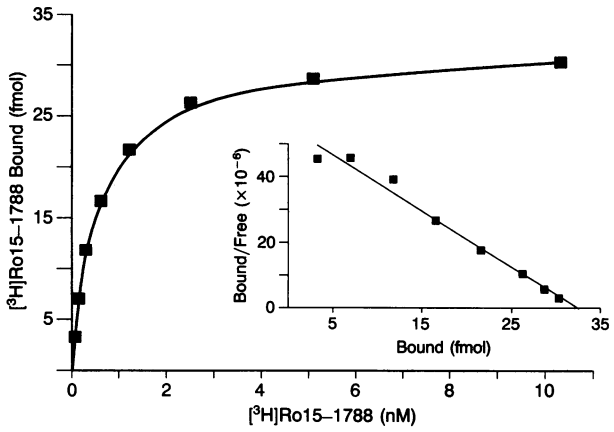

FIG. 2. Saturation isotherm of [^{3}H]Ro15-1788 binding to 1D1-PA3 cell membranes. (*Inset*) The corresponding Scatchard plot (K_d = 0.55 nM). In five independent experiments, a K_d value of 0.42 ± 0.13 nM was obtained.

version of the larger, 52-kDa β_1 band. We were not able to consistently detect a band with the anti-γ subunit antibody, even though this antiserum has high titer, as measured by immunoprecipitation of [^{3}H]Ro15-1788-labeled receptors (data not shown). It is possible that this subunit is difficult to visualize because it is susceptible to proteolysis. Indeed, we did inconsistently see a diffuse band of ≈45 kDa (data not shown) similar to that reported by Stephenson *et al.* (27).

Expressed Receptor Displays the Expected Ligand Affinities. To investigate the pharmacological characteristics of the expressed receptor, ligand-binding studies were performed with 1D1-PA3 cell membranes. Scatchard analysis of [^{3}H]Ro15-1788 binding to 1D1-PA3 cell membranes (Fig. 2) yielded a K_d value of 0.42 ± 0.13 nM. Displacement of [^{3}H]Ro15-1788 by a range of $GABA_A$ receptor ligands revealed that the cell line displays a BZ-1 pharmacology (Table 1), as previously reported for the same subtype in transient transfection systems (10, 16). Most important, the K_i values obtained were in excellent agreement with those found for anti-α_1 antibody immunoprecipitated rat brain $GABA_A$ receptors (21) for all ligands compared. The binding of these ligands could be modulated by GABA, demonstrating allosteric coupling. The efficacy of the BZ ligand was quantitated in terms of the GABA shift—that is, the ratio of the K_i values determined in the absence and presence of 100 μM GABA. As predicted from binding data for native receptor from rat brain (28), the full agonist diazepam showed a large increase in affinity in the presence of GABA. The agonist zolpidem demonstrated a slightly smaller increase in affinity, while the inverse agonist methyl β-carboline-3-carboxylate exhibited a decrease in affinity.

Recombinant $\alpha_1\beta_1\gamma_{2L}$ $GABA_A$ Receptor Macromolecule Is the Same Size as the Native Receptor. The size of the assembled recombinant receptor macromolecules was compared with that of native rat $GABA_A$ receptors by FPLC gel filtration chromatography. Under the conditions used, the $GABA_A$ receptors solubilized from rat brain and from stably transfected cells, as detected by [^{3}H]Ro15-1788 binding, migrated in a single peak between the 440-kDa and 669-kDa markers (Fig. 3). A similar result was obtained when [^{3}H]muscimol was used to assay the gel filtration fractions (data not shown). This corresponds to a Stokes radius of 7.3 nm and is almost identical to the hydrodynamic properties of the $GABA_A$ receptor purified from mammalian brain as extensively characterized previously (29). Analysis of the solubilized receptor by gel filtration is a measure of the size of the receptor–detergent complex. Under conditions where the receptor is solubilized with deoxycholate and NaCl is included to prevent aggregation of the receptor (the conditions used in this study), the contribution of the mass of detergent to the receptor–detergent complex has been previously measured, and an apparent mass of 240 kDa for the $GABA_A$ receptor protein has been determined (29).

Table 1. Affinity (K_i) and GABA-shift values of selected ligands at bovine recombinant $\alpha_1\beta_1\gamma_{2L}$ $GABA_A$ receptors labeled with [^{3}H]Ro15-1788 (0.3 nM)

	K_i, nM			
		$\alpha_1\beta_1\gamma_{2L}$ receptors from 1D1-PA3 transfected cells		
Ligand	Rat brain receptor	− GABA	+ 100 μM GABA	GABA shift
Diazepam	ND	59.65 ± 7.25	23.74 ± 3.56	2.39 ± 0.33
Zolpidem	31.36 ± 8.7	54.20 ± 2.02	30.05 ± 3.81	1.86 ± 0.34
Ro15-4513	ND	4.98 ± 0.51	5.97 ± 0.76	0.84 ± 0.03
β-CCM	1.1 ± 0.32*	1.05 ± 0.15	1.55 ± 0.19	0.66 ± 0.16
Flunitrazepam	2.54 ± 0.7*	8.90 ± 2.54	ND	ND
CL218,872	137.6 ± 36.9	129.62 ± 39.09	ND	ND
FG8205	0.85 ± 0.30*	1.02 ± 0.23	ND	ND
DMCM	14.85 ± 3.4	27.09 ± 5.58	ND	ND

Affinities determined by displacement of [^{3}H]Ro15-1788 ([^{125}I]iodoclonazepam where indicated by asterisk) from α_1-immunoprecipitated rat brain $GABA_A$ receptors (21) are included for comparison. Data are means ± SEM. β-CCM, methyl β-carboline-3-carboxylate; DMCM, methyl 4-ethyl-6,7-dimethoxy-β-carboline-3-carboxylate. ND, Not determined.

Equal proportions of [^{3}H]Ro15-1788 binding activity were immunoprecipitated with each of the protein A-Sepharose-immobilized antibodies. With crude solubilized cell membrane preparations, the anti-α_1 antibody bound 61 ± 8% (n = 4), the anti-β antibody 61 ± 5% (n = 4), and the anti-γ antibody 55 ± 4% (n = 4) of the total [^{3}H]Ro15-1788 binding sites present. In the gel filtration eluate containing the recombinant receptor, the anti-α_1 beads immunoprecipitated 83%, the anti-β beads 96%, and the anti-γ beads 91% of total [^{3}H]Ro15-1788 binding (n = 1). As these beads had been pretitrated to maximally bind all the receptors, this is further evidence that the α_1, β_1, and γ_{2L} subunits have coassembled to form a $GABA_A$ receptor macromolecule. The lower percentages immunoprecipitated from crude solubilized membranes as compared with the receptor peak eluted from the gel filtration column are probably attributable to aggregation of receptor in the former case, leading to decreased accessibility to the immobilized antibodies.

$\alpha_1\beta_1\gamma_{2L}$-Transfected Cells Express Functional $GABA_A$ Receptors with Allosteric Modulatory Sites. Some of the functional aspects and pharmacology of the recombinant receptor were characterized further by electrophysiological techniques. Application of GABA to voltage-clamped transfected cells, in experiments using the whole-cell variant of the patch-clamp technique, induced inward currents at negative holding potentials that reversed at a membrane potential of 5 ± 0.6 mV (n = 5). Logarithmic concentration–response curves for GABA were fitted with the logistic equation and gave a mean pEC_{50} of 5.2 ± 0.1, a slope factor of 1.9 ± 0.2, and a maximal response of 14.0 ± 3.7 nA (n = 4).

A concentration of 3 μM GABA was chosen to investigate the effects of allosteric modulators of GABA receptor function, as this lay at the bottom end of the fully logarithmic concentration–response curve. Stable responses to GABA (3 μM) applied at 30-sec intervals were established, and then either flunitrazepam (100 nM), alphaxalone (3 μM), or pentobarbitone (100 μM) was coapplied. All three compounds produced marked potentiations of GABA responses (Fig. 4) that were reversed upon washout. The mean percentage potentiations produced were as follows: flunitrazepam, 184 ± 43% (n = 4); alphaxalone, 235 ± 90% (n = 4); pentobarbitone, 348 ± 104% (n = 4). More detailed quantitative studies will be reported elsewhere.

DISCUSSION

Permanent cell expression systems offer a more convenient and reproducible means of receptor study than their transient equivalents. In comparisons of the responses of a number of receptor subtypes, such as the members of the $GABA_A$ receptor family, stable cell lines allow defined subtypes to be investigated in isolation. However, it is critical that each recombinant receptor produced in a cell line be shown to possess the same properties as native receptors. Toward this end, we have produced a stable cell line expressing high levels of the bovine $\alpha_1\beta_1\gamma_{2L}$ $GABA_A$ receptor subtype in response to induction with steroid. The structural and pharmacological properties of this cell line have been compared with those of native brain $GABA_A$ receptors.

The use of an inducible promoter controlling expression from the cDNAs enabled any potential toxicity problems generated by the introduction of a large number of ion channels into the cell line to be minimized. The induction system used here provided a very tight control over this

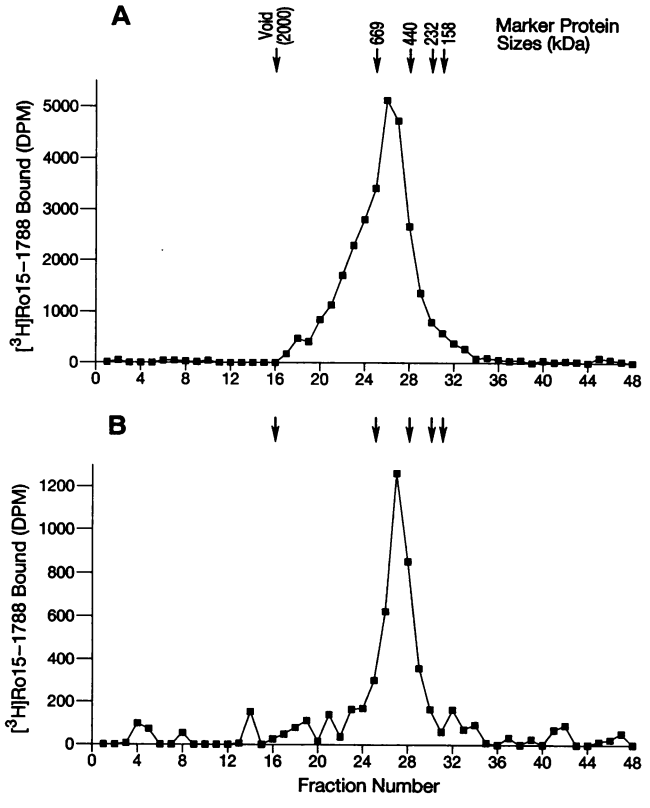

FIG. 3. Gel-exclusion chromatography of $GABA_A$ receptors solubilized from rat brain (*A*) and stably transfected 1D1-PA3 cells expressing the bovine $\alpha_1\beta_1\gamma_{2L}$ $GABA_A$ receptors (*B*). Sodium deoxycholate-extracted receptor was chromatographed (see *Materials and Methods*) and fractions were assayed for specific [^{3}H]Ro15-1788 binding. Size markers are indicated by arrows.

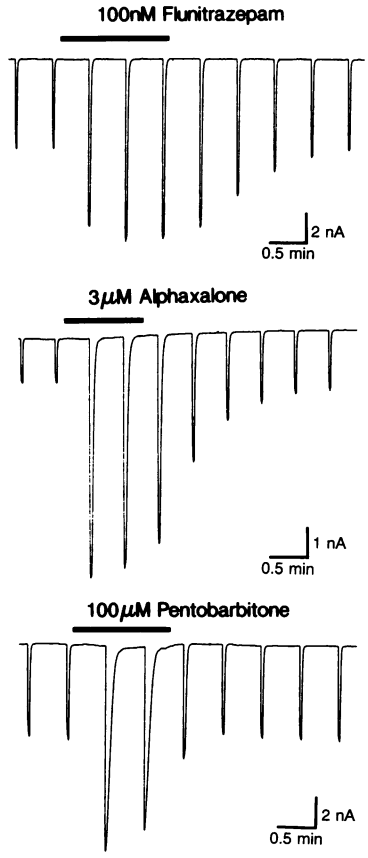

FIG. 4. Functional analysis of the stably expressed bovine $\alpha_1\beta_1\gamma_{2L}$ $GABA_A$ receptor by the whole-cell patch-clamp technique. Downward deflections represent inward chloride currents produced following the application of 3 μM GABA alone, or together with the indicated drugs. Bars indicate the duration of drug application.

expression, with no evidence of expression of any of the subunit mRNAs in the absence of the dexamethasone inducer. However, in the presence of dexamethasone, there was a relatively rapid induction of receptor synthesis, which resulted in the expression of a large number of receptors. Although a stable cell line expressing an $\alpha_1\beta_1$ $GABA_A$ receptor has been reported (30), it has been of somewhat limited use as the level of expression was relatively poor (200 [^{3}H]muscimol binding sites per cell) and the lack of a γ subunit compromised its usefulness as a model of native $GABA_A$ receptors, since there is little evidence that $\alpha\beta$ combinations exist *in vivo* without additional subunits of the γ or δ class (21, 31).

To establish whether or not such cell lines are good model systems for the investigation of the size, assembly, and structure of $GABA_A$ receptor macromolecules, gel filtration analysis was performed. Comparison of the profiles of the native receptor from rat brain and the receptor expressed in the 1D1-PA3 cells indicated that both had the same Stokes radius and were probably of the same size. This implies that the receptor expressed by the transfected cells is composed of the same number of subunits as native $GABA_A$ receptors and further that, since the Stokes radius is the same, it adopts the same basic shape. However, the native receptor profile includes a shoulder of higher molecular weight material attached to the major peak, which is not present in that generated for the recombinant receptor. One possible explanation may be that a minority of receptors from rat brain are solubilized with additional proteins present in the complex. A similar shoulder was observed by Mamalaki *et al.* (29) when receptor from bovine brain was solubilized under similar conditions. These additional proteins must be loosely attached, since no shoulder was observed with purified native brain receptor (29).

By analogy with other members of the ligand-gated ion channel family, it is generally assumed that functional receptors are composed of five subunits. The logarithmic concentration–response curves for GABA obtained from the patch-clamp experiments yielded a slope factor close to 2. This suggests that GABA must bind to at least two of these subunits for channel activation and that cooperativity of binding may also occur. Although we have demonstrated that the stably transfected cells produce a receptor of the same size and shape as native rat brain receptors, no information is provided by these studies as to the stoichiometry of the α_1, β_1, and γ_{2L} subunits in the receptor macromolecule, nor to which of these subunits the agonist binds to evoke a response in functional studies. However, our quantitative immunoprecipitation studies indicate the likely coassembly of α, β, and γ subunits in such cell lines.

If stably transfected cell lines are to be of use in the study of the pharmacologies of different ligands at the subtype receptor, it is essential that the affinities and efficacies of the ligands are the same at both recombinant and native sites. Within the range of the ligands tested, the pharmacological binding data produced are in excellent agreement with those obtained previously, both in comparison with transient expression systems (10, 16) and with immunoprecipitated rat brain receptors (21). Further, the functional studies demonstrated that GABA responses mediated by the recombinant receptor were potentiated by BZs, steroids, and barbiturates, which are believed to act at distinct sites on native $GABA_A$ receptors to produce their allosteric modulation.

These results indicate that the stably transfected cell line 1D1-PA3, described here, expresses high levels of the $\alpha_1\beta_1\gamma_{2L}$ $GABA_A$ receptor subtype and that the expressed receptor possesses structural, ligand-binding, and functional properties similar to those a native receptor of the same subtype. Thus we propose that such cell lines will act as useful model systems for the analysis of the structures and pharmacologies of individual members of this complex family of neurotransmitter receptors.

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