Assembly of GABA_A Receptor Subunits: Role of the δ Subunit

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GABA_A receptor channels (GABARs) composed of different combinations of rat $\alpha 1$, $\beta 1$, $\gamma 2L$, and δ subunits were expressed transiently in mouse fibroblast cells (L929 cells). Whole-cell recordings were obtained from transfected cells to determine which combinations of GABAR subunits formed functional receptor channels, and to compare the electrophysiological and pharmacological characteristics of GABAR channels expressed in the presence and absence of the δ subunit. Only $\alpha 1\beta 1\gamma 2L$, $\alpha 1\beta 1\gamma 2L\delta$, and $\alpha 1\beta 1\delta$ subunit combinations assembled to form functional GABAR channels and the presence of the δ -subunit slowed the rate of acute desensitization of GABA-evoked current during GABA application and the rate of recovery of GABA-evoked current following GABA application. These three different GABAR channel isoforms also showed distinct pharmacological profiles with differential sensitivity to block by zinc. Zinc was a potent blocker of $\alpha 1\beta 1\delta$ GABAR channels, a moderate-strength blocker of $\alpha 1\beta 1\gamma 2L\delta$ GABAR channels, and did not block the $\alpha 1\beta 1\gamma 2L$ GABAR channels. These findings suggest that GABAR isoforms containing the δ subunit constitute a novel GABAR channel with distinct electrophysiological and pharmacological characteristics.

[Key words: GABA_A receptor, patch clamp, recombinant DNA, transient expression, δ subunit, cations]

The GABA_A receptor (GABAR) in the CNS is a heteroligomeric protein complex. It consists of an integral GABA-gated Cl channel with distinct binding sites for allosteric modulators including benzodiazepines, β -carbolines, barbiturates, neurosteroids, and picrotoxin. The native GABAR is probably a pentamer (Stephenson, 1988) composed of several different subunits belonging to at least five distinct classes, α , β , γ , δ , and ρ . To date, 15 different GABAR subunit subtypes (six α , three β , three γ , one δ , and two ρ) have been identified by molecular cloning (Schofield et al., 1987; reviewed in Macdonald and Olsen, 1994). The δ subunit is one of the most recent to be cloned (Shivers et al., 1989), and members within each class show 60–80% sequence identity while 20–40% sequence identity exists between the different classes (Levitan et al., 1988; Shivers et al., 1989; Olsen and Tobin, 1990). The various GABAR subunits share a common structural motif which consists of four putative transmem-

brane segments (M1–M4), a large NH₂-terminal signal peptide featuring a disulfide-bonded β -structural loop and a long variable intracellular domain between M3 and M4. While GABAR isoforms composed of α , β , and γ subunits have been studied, relatively little is known about the electrophysiological and pharmacological properties of GABAR isoforms containing the δ subunit. *In situ* hybridization studies in rat brain suggest that the distribution of the δ subunit in the brain resembles that of the high affinity GABA_A receptor labeled with ³H-muscimol (Benke et al., 1991; Laurie et al., 1992). The δ subunit mRNA is expressed most prominently in the cerebellum and at lower levels in the thalamic nuclei, dentate gyrus, olfactory bulb, olfactory tubercle, cerebral cortex, and nucleus accumbens. A similar distribution pattern for the δ subunit has been reported by immunohistochemical mapping of GABAR subtypes containing the δ subunit in the rat brain (Benke et al., 1991). In the hippocampal dentate gyrus, granule cell δ subunit mRNA colocalizes with $\alpha 1$, $\beta 1$, and $\gamma 2L$ subunit mRNAs while in the cerebellum it colocalizes with $\alpha 1$, $\alpha 6$, β , and $\gamma 2$ subunit mRNAs (Laurie et al., 1992). Using antiserum specific for the δ subunit, Mertens et al. immunoprecipitated a population of GABARs containing high affinity benzodiazepine binding sites from rat brain extracts (Mertens et al., 1993). Immunoaffinity chromatography of the purified receptor using the δ subunit antiserum showed the δ subunit to be associated with $\alpha 1$, $\alpha 3$, $\beta 2$, $\beta 3$, and $\gamma 2$ subunits. In a separate study by Ragan et al., analysis of the subunit composition of GABARs in the rat cerebellum using mixtures of two subunit-specific antibodies revealed that the majority of the receptors contained only one α subtype while the γ and δ subunits were mutually exclusive (Ragan et al., 1993). Their results indicated good agreement between binding data for GABAR ligands in cells transfected with cloned subunits and results on immunopurified receptors from rat brain, providing valuable confirmation that the pharmacology of GABARs in heterologous expression experiments is the same as that in the brain. However, the role of the δ subunit in GABAR function has remained uncertain.

The aim of the present study was to compare the electrophysiological and pharmacological properties of recombinant GABARs in the absence and presence of the δ subunit in mouse fibroblast L929 cells cotransfected with different combinations of $\alpha 1$, $\beta 1$, $\gamma 2L$, and δ subunits. Recent work by Angelotti et al. (1993) using L929 cells has demonstrated that not all combinations of GABAR subunits readily assemble into functional GABARs and that there are certain preferred forms of the mature GABAR based on the types of subunits present in these cells. They reported that of the various double- and triple-subunit combinations of $\alpha 1$, $\beta 1$, and $\gamma 2S$ subunits expressed in L929 cells, only $\alpha 1\beta 1$ and $\alpha 1\beta 1\gamma 2S$ but not $\alpha 1\gamma 2S$ or $\beta 1\gamma 2S$

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GABARs were functionally expressed. They also proposed that if no whole-cell responses were seen with certain double-subunit combinations, then assembly of functional GABARs from either single subunit would not have occurred, thus eliminating the possibility of homomeric $\alpha 1$, $\beta 1$, and $\gamma 2S$ GABAR channels. The present study also suggests that there are certain preferred forms of functional GABARs expressed in L929 cells when the δ subunit is added to or substituted for the $\alpha 1$, $\beta 1$, or $\gamma 2L$ subunits.

Materials and Methods

Sources. FDG and Imagene were purchased from Molecular Probes, Inc. (Eugene, OR). All other chemicals, drugs, sera, media, and cell attachment factors were obtained from GIBCO-Bethesda Research Labs (Bethesda, MD), Boehringer Mannheim Biochemicals (Indianapolis, IN), or Sigma Chemicals (St. Louis, MO). Tissue culture dishes were purchased from Corning Glass Works (Corning, NY). All cell lines were purchased from ATCC (Rockford, MD). Restriction and DNA modification enzymes were obtained from either Bethesda Research Labs, Boehringer Mannheim Biochemicals, or New England Biolabs, Inc. (Beverly, MA). Diazepam was kindly provided by Hoffmann-LaRoche (Nutley, NJ).

Plasmid construction. Full-length cDNAs encoding the rat $\alpha 1$, $\beta 1$, $\gamma 2L$, and δ subunits of the GABAR were kindly provided by Dr. A. J. Tobin ($\alpha 1$ and $\beta 1$; UCLA) and Dr. K. Angelides (δ ; Baylor College of Medicine, Texas) in the bluescript vector while $\gamma 2L$ was cloned in our lab by Fang Tan (University of Michigan). The bovine and rat cDNAs have been described previously ($\alpha 1$, $\beta 1$, $\gamma 2L$; Schofield et al., 1987; Zaman et al., 1992; δ ; Shivers et al., 1989). All plasmids were cut with appropriate restriction enzymes to release the complete open reading frames and approximately 10–100 base pairs of the 5' and 3' untranslated regions, including the Kozak sequences (Kozak, 1981, 1984) and were subcloned individually into the BglII site of the mammalian expression vector pCMVNeo (Hugenvik, 1991) to form the plasmids pCMV $\alpha 1$, pCMV $\beta 1$, pCMV $\gamma 2L$, and pCMV δ . The vector pCMV β Gal was created by subcloning a 3000 base pair BglII fragment of pSV β Gal (Hall et al., 1983) (from Dr. Audrey Seasholtz, University of Michigan) into pCMVNeo.

Preparation of gridded plates. A Mecnex BB Form 2 device (Medical Systems, Inc. Greenvale, NY) was used to imprint a 26 $\times$ 26 grid (300 μ m per grid edge) on the bottom of a 35 mm tissue culture dish, according to the manufacturer's instructions. Individual grid spaces are identified by a corresponding two letter alphabetic code. After plating at low density, cells could be accurately located on the dish relative to a particular grid while switching between the fluorescent and electrophysiology microscopes. The process of imprinting the grid removed some of the net negative charge on the dish necessary for cell adherence; thus, some cell lines tested required the plates to be coated with adhesion factors. A coating of one or two drops of collagen (0.5 mg/ml) in phosphate-buffered saline was used to obtain optimal cell adherence.

Cell culture and DNA transfection. All cell lines were grown in DMEM with 100 IU/ml of penicillin and 100 μ g/ml of streptomycin, at 37°C in 5% CO₂, 95% air. L929 cells were grown with 10% horse serum. For transfections, cell lines were passaged the night before with trypsin/EDTA solution (0.5%, 0.2%, respectively) and plated at approximately 70% confluency in a 60 mm dish. The next day, cells were transfected using the modified calcium phosphate precipitation method (Chen and Okayama, 1987) with various combinations of CsCl banded pCMV $\alpha 1$, pCMV $\beta 1$, pCMV $\gamma 2L$, pCMV δ , and pCMV β Gal. Plasmids were mixed in a 1:1:1:1:1 (α : β : γ : δ : β Gal) or 1:1:1:1 (α : β : γ : β Gal or α : β : δ : β Gal) ratio while maintaining the total amount of DNA added per 60 mm dish at 9.6 μ g in 300 μ l of transfection buffer. Cells were shocked with a 15% glycerol/1 $\times$ PBS solution 4 or 5 hr later. Twenty-four hours after the addition of the precipitate, cells were passaged as above, placed into sterile 15 ml conical tubes, and treated with 350 μ g/ml tissue culture grade DNase I for 10 min at 37°C. After pelleting the cells at 400 $\times$ g, they were plated onto either standard 35 mm or Mecnex-gridded plates. Electrophysiological analysis was performed 24 hr later.

β -Galactosidase staining protocols. Two methods of β -galactosidase staining were utilized. 5-bromo-4-chloro-3-indoyl β -D-galactoside (X-Gal) staining of cells was performed as described previously (Sanes et al., 1986) to determine the transfection efficiency. FDG staining was performed as originally described (Nolan et al., 1988) with the following

slight modifications made for use with adherent cells. Cells were washed twice in PBS to remove the medium and then incubated for 5 min at 37°C with 1 ml of PBS to reequilibrate the cells to this temperature. During the incubation step, 20 mM FDG solution (prepared by the manufacturer) was diluted 1:20 (25 μ l) into 0.5 ml of 0.5 $\times$ PBS in a microcentrifuge tube, and placed in a 37°C water bath. The PBS was aspirated from the cells, and the warmed 1 mM FDG solution (final concentration) was added to the cells. The plate was warmed in the 37°C water bath for 1 min, then placed on ice, and 2.5 ml of ice-cold 1 $\times$ PBS was added. After 5 min on ice, the cells were viewed with a fluorescence microscope fitted with fluorescein filters. Imagene staining for lacZ activity was performed according to the manufacturer's instructions.

Electrophysiological analysis. Prior to recording, the PBS/FDG solution on the plate of cells was exchanged with five 2 ml washings of external recording medium containing the following, in mM: 142 NaCl, 8.1 KCl, 6 MgCl₂, 1 CaCl₂, 10 glucose, 10 HEPES (pH $\sim$ 7.4). The intrapipette solution contained, in mM, 153 KCl, 1 MgCl₂, 5 EGTA, 10 HEPES (pH $\sim$ 7.3). This combination of external and intrapipette solutions produced a chloride equilibrium potential (E_{Cl}) of approximately 0 mV and a potassium equilibrium potential (E_K) of -75 mV across the patch membrane. GABA, diazepam, pentobarbital, and zinc chloride were diluted with external recording solution from a stock solution (10 mM in water and 0.41 mM in DMSO and water for diazepam) to the indicated final concentration on the day of the experiment. Drugs were applied by a pressure ejection micropipette (10–15 μ m tip diameter; 1.0–1.5 psi) placed next to the cell or patch. Micropipettes and recording electrodes were fabricated on a Flaming Brown micropipette puller (Sutter Instruments Co., model P-87). Recording electrodes were coated with Q-Dope before use and had resistances ranging from 5 to 10 M Ω when filled with the internal solution and immersed in a dish containing the external solution. Whole-cell current recording was performed with methods described previously for mouse spinal cord neuron recordings (Macdonald et al., 1989a; Porter et al., 1990) using a List L/M EPC-7 amplifier (Darmstadt, Germany). All recordings were done at room temperature (22–24°C).

Whole-cell and single-channel data collection and analysis. Currents were recorded simultaneously on a videocassette recorder (Sony SL-HF360) via a digital audio processor (Sony PCM-501 ES, 14-bit, 44 kHz) and a chart recorder (Gould Inc., Cleveland, OH) for later computer analysis. Whole-cell and single-channel recordings were low-passed filtered (3 dB at 1 kHz, 8-pole Bessel filter, Frequency Devices) before the chart recorder. The peak whole-cell current amplitude was measured by hand from the chart output. Single-channel records were analyzed using methods described previously for mouse spinal cord neurons (Macdonald et al., 1989a; Porter et al., 1990).

Results

GABA responsiveness of GABARs containing the δ subunit

To determine which combinations of rat $\alpha 1$, $\beta 1$, $\gamma 2L$, and δ subunits could assemble in L929 cells to give functional GABARs, we studied cells transfected with GABAR subunit cDNA expression vectors encoding the δ subunit alone (homomeric), $\delta\beta 1\gamma 2L$, $\alpha 1\beta 1\gamma 2L$, and $\alpha 1\beta 1\delta$ subunits (trimeric), and $\alpha 1\beta 1\gamma 2L\delta$ subunits (tetrameric). Angelotti et al. (1993) have recently demonstrated that no functional homomeric GABARs composed of $\alpha 1$, $\beta 1$, or $\gamma 2S$ subunits or dimeric GABARs composed of $\beta 1\gamma 2S$ or $\alpha 1\gamma 2S$ subunits are expressed in L929 cells transfected with the relevant subunits. Based on the premise that subunits which assembled together in nature would have a higher affinity to associate with each other, and therefore, show a higher efficiency of expression in L929 cells, we identified combinations of subunits that showed high efficiency of expression of functional GABARs. The number of cells with GABA-evoked responses was determined by recording whole-cell currents from FDG-positive cells with application of 10–100 μ M GABA (Angelotti and Macdonald, 1993; Angelotti et al., 1993). Table 1 lists the fraction of cells cotransfected with the different subunit combinations which were responsive to 10 or 100 μ M GABA. Cells transfected with $\alpha 1\beta 1\gamma 2L$ subunit cDNAs showed GABA-

Figure 1. Representative whole-cell currents from cells transfected with different combinations of $\alpha 1$, $\beta 1$, $\gamma 2L$, and δ subunits. Whole-cell currents were recorded from individual cells transfected with either δ alone, $\delta\beta 1\gamma 2L$, $\alpha 1\delta\gamma 2L$, $\alpha 1\beta 1\delta$ (1a); $\alpha 1\beta 1\gamma 2L$ or $\alpha 1\beta 1\gamma 2L\delta$ (1b) subunit cDNAs. Each cell was voltage clamped at a potential of -75 mV and a 5 sec pulse of $10 \mu M$ GABA was applied to elicit a response. The bars above the current traces indicate the duration of application of GABA. The inward current (downward deflection) denotes outward movement of the negatively charged chloride ions at -75 mV ($E_{Cl} = 0$ mV).

evoked responses in 58% of the cells studied similar to earlier reports (Angelotti et al., 1993). Cells transfected with δ subunit cDNA alone did not respond to GABA. To determine if the δ subunit could substitute for $\alpha 1$, $\beta 1$, or $\gamma 2L$ subunits, we studied trimeric subunit combinations with the δ subunit replacing either the $\alpha 1$, $\beta 1$, or $\gamma 2L$ subunits. Cells transfected with δ , $\beta 1$ and $\gamma 2L$ subunit cDNAs showed no GABA responsiveness while those transfected with $\alpha 1$, δ , and $\gamma 2L$ subunit cDNAs or $\alpha 1$, $\beta 1$, and δ subunit cDNAs showed a low percentage of cells that responded to GABA (11% and 18%, respectively). This suggests that the δ subunit does not form homomeric GABAR channels, does not substitute for the $\alpha 1$ subunit and shows a low efficiency of expression to form functional GABAR channels when used to substitute for $\beta 1$ or $\gamma 2L$ subunits in L929 cells (Fig. 1A). However, addition of δ subunit to $\alpha 1\beta 1\gamma 2L$ subunits resulted in 50% of the cells studied being GABA responsive, suggesting that this combination of subunits showed a higher efficiency of expression than the combinations where δ was used to substitute for any of the other three subunits (Fig. 1B).

Therefore, based on the above findings, we concluded that the three most likely combinations of $\alpha 1$, $\beta 1$, $\gamma 2L$, and δ subunits that occur in nature to form GABAR isoforms and would be important to characterize electrophysiologically and pharmacologically were the $\alpha 1\beta 1\gamma 2L$, $\alpha 1\beta 1\delta$, and $\alpha 1\beta 1\gamma 2L\delta$ subunit combinations.

Comparison of GABAR whole-cell currents in the absence and presence of the δ subunit

To determine the role of the δ subunit in functional GABARs, we compared the effects of substitution of the δ subunit for the $\gamma 2L$ subunit and that of addition of the δ subunit to $\alpha 1\beta 1\gamma 2L$ combination. Specifically, we studied the time course of recovery of GABA-evoked currents and the peak amplitudes of the whole-cell currents. $\alpha 1\beta 1\gamma 2L$ GABAR whole-cell currents showed desensitization during the course of GABA application, which resulted in a sharp peak at the maximum value of the GABA-evoked current, and a rapid recovery of the whole-cell

current to baseline value following GABA application (Fig. 2). In contrast, $\alpha 1\beta 1\delta$ GABAR whole-cell currents displayed little desensitization during the course of GABA application and a very slow recovery to baseline following GABA application. $\alpha 1\beta 1\gamma 2L\delta$ GABAR whole-cell currents showed characteristics that were intermediate between $\alpha 1\beta 1\gamma 2L$ and $\alpha 1\beta 1\delta$ GABAR currents. To provide a more quantitative estimate of the differences in the rates of desensitization during the application of GABA and the rates of recovery of whole-cell currents evoked by GABA, the time course of decay of the $\alpha 1\beta 1\gamma 2L$, $\alpha 1\beta 1\gamma 2L\delta$ and $\alpha 1\beta 1\delta$ GABAR currents during the application of $10 \mu M$ GABA and the time course for the currents to recover to predrug or baseline values following the end of GABA application were fitted with single exponential functions (SIGMAPLOT version 5.0). The time constant of the exponential function fit to the current during GABA application (beginning from peak of the current to the current level at end of GABA pulse) provided an estimate of the rate of desensitization (τ_d) while that of the fit to the current following end of GABA application (beginning from end of GABA pulse to recovery of current to pre-GABA value)

Table 1. Correlation of GABAR expression with FDG-positive cells

Subunit combinations	Fraction of cells responsive to GABA	% GABA responsive
δ	0/18	0
$\delta\beta 1\gamma 2L$	0/18	0
$\alpha 1\delta\gamma 2L$	2/18	11
$\alpha 1\beta 1\delta$	20/114	18
$\alpha 1\beta 1\gamma 2L$	35/60	58
$\alpha 1\beta 1\gamma 2L\delta$	48/97	50

Cells were transfected with different combinations of GABAR subunit cDNAs. A fluorescent β -galactosidase (FDG) assay (Angelotti et al., 1993) was used to identify successfully transfected (LacZ-positive) cells. The fraction of LacZ-positive cells that displayed currents evoked by 10 or $100 \mu M$ GABA was determined to estimate the efficiency of expression of the various combinations of GABAR subunits.

Figure 2. Differences in the rate of desensitization of GABA-evoked whole-cell currents during application of GABA. Currents elicited at $V_h = -75$ mV during a 5 sec pulse of 10 mM GABA are shown on an expanded time scale. Horizontal bars denote the duration of GABA application.

provided an estimate of the rate of recovery (τ_r) of GABA-evoked current. GABA-mediated desensitization of whole-cell currents was characterized by τ_d while the dissociation of ligand (GABA) from its receptor was related to τ_r . The average value of τ_d (mean $\pm$ SEM) for $\alpha 1\beta 1\gamma 2L$ GABAR currents was 23.58 ± 3.5 sec ($n = 14$) while that for $\alpha 1\beta 1\gamma 2L\delta$ GABAR currents

was 57.3 ± 11.8 sec ($n = 17$). There were some $\alpha 1\beta 1\gamma 2L\delta$ GABAR currents that did not show any decay during GABA application, and therefore, could not be fit with exponential functions to determine τ_d . The difference between τ_d for these two different GABAR channels were statistically significant based on independent Student's t test ($P < 0.02$). The average times (mean $\pm$ SEM) for the GABA-evoked $\alpha 1\beta 1\gamma 2L$, $\alpha 1\beta 1\gamma 2L\delta$, and $\alpha 1\beta 1\delta$ GABAR currents to decay to baseline (τ_r) were 6.6 ± 0.8 sec ($n = 23$), 8.3 ± 0.7 sec ($n = 37$) and 16.9 ± 1.9 sec ($n = 6$), respectively. The differences between the τ_r values were also statistically significant ($P < 0.00003$) except for that between $\alpha 1\beta 1\gamma 2L$ and $\alpha 1\beta 1\gamma 2L\delta$ GABAR currents ($0.05 < P < 0.1$). The drug application system provided drug delivery time to maximum concentration of less than 1 sec, which was consistent and reproducible for all the subunit combinations examined here.

The average amplitudes of $\alpha 1\beta 1\gamma 2L$ and $\alpha 1\beta 1\gamma 2L\delta$ GABAR whole-cell currents [1540 ± 693 pA (mean $\pm$ SEM, $n = 5$) and 667 ± 321 pA (mean $\pm$ SEM, $n = 6$), respectively] evoked by 10 μ M GABA were 30–75 times larger than the amplitude of $\alpha 1\beta 1\delta$ GABAR currents [41.1 ± 14 pA (mean $\pm$ SEM, $n = 11$)] (Fig. 3). Concentration–response (CR) curves for GABA concentrations ranging from 1 to 100 μ M for the three subunit combinations were obtained (Fig. 4). The CR curves for $\alpha 1\beta 1\gamma 2L$ and $\alpha 1\beta 1\gamma 2L\delta$ GABAR channels were sigmoidal in shape with Hill coefficients of 1.4 and 2.2 while the $\alpha 1\beta 1\delta$ GABAR channels displayed a very shallow CR curve. The values of the half-maximum concentration (EC_{50}) of GABA for $\alpha 1\beta 1\gamma 2L$ and $\alpha 1\beta 1\gamma 2L\delta$ GABAR currents were 6.2 μ M and 6.9 μ M, respectively.

Effects of diazepam, pentobarbital, and Zn^{2+} on $\alpha 1\beta 1\delta$, $\alpha 1\beta 1\gamma 2L$, and $\alpha 1\beta 1\gamma 2L\delta$ GABAR currents

To distinguish among GABARs in cells cotransfected with the three different subunit combinations, we used pharmacological

Figure 3. Whole-cell currents elicited by increasing concentrations of GABA from cells transfected with $\alpha 1\beta 1\gamma 2L$, $\alpha 1\beta 1\gamma 2L\delta$, and $\alpha 1\beta 1\delta$ subunits. Cells were voltage clamped at -75 mV and 5 sec pulses of the indicated concentrations of GABA were applied. Horizontal bars indicate the duration of GABA application.

Figure 4. Concentration-response curves for cells transfected with $\alpha 1\beta 1\gamma 2L$, $\alpha 1\beta 1\gamma 2L\delta$, and $\alpha 1\beta 1\delta$ subunit cDNAs. Symbols represent the mean value of current from six, five, and two experiments, respectively, while the error bars represent the SEM.

agents known to have distinct actions in the presence or absence of specific subunits. The first drug examined was the benzodiazepine, diazepam. The action of diazepam depends on the subunit composition of GABARs. It increases the amplitude of GABA-evoked currents recorded from GABARs containing the γ subunit by shifting the concentration-response curve for GABA to the left and thus decreasing the value of EC_{50} (Horne et al., 1993). However, it does not enhance GABAR currents in GA-

BARs that lack the γ subunit. The effects of 100 nM diazepam in the presence of 10 or 100 μM GABA on $\alpha 1\beta 1\gamma 2L$, $\alpha 1\beta 1\gamma 2L\delta$, and $\alpha 1\beta 1\delta$ GABAR currents were studied (Fig. 5). Application of diazepam did not increase the $\alpha 1\beta 1\delta$ GABAR currents elicited by 10 or 100 μM GABA, consistent with the absence of the γ subunit in this combination. In contrast, $\alpha 1\beta 1\gamma 2L\delta$ and $\alpha 1\beta 1\gamma 2L$ GABAR currents were enhanced significantly in the presence of diazepam as compared to the currents elicited by GABA alone. The average values for the percentage enhancement of the $\alpha 1\beta 1\gamma 2L\delta$ and $\alpha 1\beta 1\gamma 2L$ GABAR currents were 60 ± 21 and $38 \pm 19\%$ (mean $\pm$ SEM, $n = 3$), respectively.

The second drug studied was pentobarbital. Barbiturates enhance GABAR currents irrespective of their subunit composition and are postulated to act by modifying GABAR channel gating (Macdonald et al., 1989b). Pentobarbital (10 μM) enhanced currents evoked by 10 or 100 μM GABA for the three different subunit combinations (Fig. 6). The average enhancement (mean) with 10 μM pentobarbital was 65% ($n = 2$), 112% ($n = 2$), and 31.5% ($n = 2$) for $\alpha 1\beta 1\gamma 2L$, $\alpha 1\beta 1\gamma 2L\delta$, and $\alpha 1\beta 1\delta$ GABAR currents, respectively. This is consistent with the ability of barbiturates to enhance GABAR currents regardless of subunit composition.

Zinc (10 μM) has been shown to reduce GABAR currents in recombinant receptors which lack the γ subunit by 80–90% but has no significant effect on GABARs that contain the γ subunit (Draughn et al., 1990). Therefore, we tested the effect of zinc (Zn^{2+}) on the three different GABAR isoforms. The $\alpha 1\beta 1\delta$ GABAR currents were blocked by 10 μM Zn^{2+} with an average decrease of 75% ($n = 2$) while the $\alpha 1\beta 1\gamma 2L$ GABAR currents were enhanced an average of $17 \pm 6\%$ (mean $\pm$ SEM, $n = 5$) in the presence of 10 μM Zn^{2+} (Fig. 7). This is consistent with the notion that the presence of the γ subunit confers insensitivity to block of GABAR current by Zn^{2+} . Interestingly, 10 μM Zn^{2+} caused a significant reduction in the $\alpha 1\beta 1\gamma 2L\delta$ GABAR currents despite the presence of the γ subunit. The average decrease in $\alpha 1\beta 1\gamma 2L\delta$ GABAR current by 10 μM Zn^{2+} was $25 \pm 4.2\%$ (mean

Figure 5. Regulation of whole-cell currents by diazepam. Currents evoked at -75 mV by 10 or 100 μM GABA are shown before, during, and after an application of 100 nM diazepam. The horizontal bars indicate the duration of GABA application.

Figure 6. Enhancement of whole-cell currents by pentobarbital. Currents elicited by 10 or 100 μ M GABA are shown before, during, and after application of 10 μ M pentobarbital. The cell was held at -75 mV, and the duration of application of GABA is indicated by the horizontal bars.

$\pm$ SEM, $n = 5$), while that with 100 μ M Zn^{2+} was $51 \pm 3.5\%$ ($n = 3$). Thus, 10 μ M Zn^{2+} exerts differential effects on $\alpha 1\beta 1\gamma 2L$, $\alpha 1\beta 1\gamma 2L\delta$, and $\alpha 1\beta 1\delta$ GABAR whole-cell currents.

Effect of lanthanum on $\alpha 1\beta 1$ and $\alpha 1\beta 1\delta$ GABAR currents

To confirm that the δ subunit was indeed expressed in cells cotransfected with $\alpha 1$, $\beta 1$, and δ subunits, rather than dimers of

$\alpha 1\beta 1$, we examined the effect of polyvalent cation lanthanum (La) on cells transfected with $\alpha 1$ and $\beta 1$ subunits (dimers) and those transfected with $\alpha 1$, $\beta 1$, and δ subunits. Lanthanum (20–300 μ M) has been shown to potentiate GABAR currents in recombinant GABAR isoforms containing α and β subunits and α , β , and γ subunits expressed in HEK293 cells (Im et al., 1992) and in dorsal root ganglion neurons (Ma et al., 1993) by reducing

Figure 7. Modulation of whole-cell currents by 10 μ M zinc. Currents evoked by 10 or 100 μ M GABA are shown before, during, and after application of 10 μ M Zn. The holding potential was -75 mV, and the duration of application of GABA is indicated by the horizontal bars.

Figure 8. Modulation of $\alpha 1\beta 1$ and $\alpha 1\beta 1\delta$ whole-cell currents by lanthanum. Currents evoked by 10 μM GABA are shown before, during, and after application of 300 μM lanthanum. Cells were held at a voltage of -75 mV, and duration of application of GABA is indicated by the horizontal bars.

the half-maximal concentration (EC_{50}) for GABA. Im et al. have reported a maximum enhancement of 1 μM GABA-evoked $\alpha 1\beta 2$ whole-cell current to 170% of control with 800 μM lanthanum. Therefore, we tested whether La could be used as a pharmacological tool to distinguish between $\alpha 1\beta 1$ and $\alpha 1\beta 1\delta$ GABAR isoforms. La at 300 μM enhanced $\alpha 1\beta 1$ currents evoked by 10 μM GABA (146% of control, $n = 2$) but did not increase the $\alpha 1\beta 1\delta$ currents evoked by 10 μM GABA (no increase; $n = 3$) (Fig. 8). Therefore, the addition of δ subunit to α and β subunits renders the GABA-evoked $\alpha 1\beta 1\delta$ currents insensitive to potentiation by lanthanum, thus distinguishing between $\alpha 1\beta 1$ and $\alpha 1\beta 1\delta$ whole-cell currents.

Comparison of $\alpha 1\beta 1\gamma 2\text{L}$, $\alpha 1\beta 1\gamma 2\text{L}\delta$ and $\alpha 1\beta 1\delta$ GABAR single-channel currents

To determine if the partial block of $\alpha 1\beta 1\gamma 2\text{L}\delta$ current by zinc was due to a subpopulation of $\alpha 1\beta 1\delta$ receptors that were assembled in cells cotransfected with $\alpha 1$, $\beta 1$, $\gamma 2\text{L}$, and δ cDNAs, characteristics of single-channel currents from cells transfected with $\alpha 1\beta 1\gamma 2\text{L}$, $\alpha 1\beta 1\gamma 2\text{L}\delta$ and $\alpha 1\beta 1\delta$ subunits were studied (Fig. 8). Initial analysis indicate that the conductance of $\alpha 1\beta 1\gamma 2\text{L}$ and $\alpha 1\beta 1\gamma 2\text{L}\delta$ GABAR single channels was comparable (30 and 33 pS, respectively) while the $\alpha 1\beta 1\delta$ GABAR channels showed a smaller conductance of 22 pS. The $\alpha 1\beta 1\gamma 2\text{L}$ and $\alpha 1\beta 1\gamma 2\text{L}\delta$ GABAR single-channels showed distinct differences in the mean open times for the single channels with values of 5 msec and 20 msec, respectively. Though the presence of brief $\alpha 1\beta 1\gamma 2\text{L}$ -like openings embedded in the longer duration openings of $\alpha 1\beta 1\gamma 2\text{L}\delta$ GABAR single channels cannot be ruled out, the majority of the $\alpha 1\beta 1\gamma 2\text{L}$ GABAR currents appeared to be carried via the 20 msec $\alpha 1\beta 1\gamma 2\text{L}\delta$ GABAR channels. The $\alpha 1\beta 1\delta$ GABAR channels showed a much longer mean open time of 400 msec with virtually no brief closings compared to $\alpha 1\beta 1\gamma 2\text{L}$ and $\alpha 1\beta 1\gamma 2\text{L}\delta$ GABAR channels. Thus, $\alpha 1\beta 1\gamma 2\text{L}$, $\alpha 1\beta 1\gamma 2\text{L}\delta$, and $\alpha 1\beta 1\delta$ GABAR channels manifest distinct single-channel characteristics.

Discussion

The δ subunit defines novel GABAR subtypes

GABA_A receptors exist as heteroligomers composed of two to four different polypeptides with a total of four or five subunits. The assembly of various combinations of these subunits in different regions of the brain could contribute to the diversity of GABAR function in the CNS (Macdonald and Olsen, 1994). As the subtypes differ not only in distribution but also in physiological function and biological regulation, they will also differ

in susceptibility to disease processes and pharmacological intervention. Since *in situ* hybridization studies indicate the colocalization of $\alpha 1$, $\beta 1$, $\gamma 2\text{L}$, and δ subunits in hippocampal dentate gyrus granule cells, in the present study, we determined the properties of recombinant GABARs expressed in L929 cells cotransfected with the above mentioned subunits. Specifically, we examined the electrophysiological and pharmacological properties of functional GABARs in the presence and absence of the δ subunit. $\alpha 1\beta 1\gamma 2\text{L}$ and $\alpha 1\beta 1\gamma 2\text{L}\delta$ were the subunit combinations that were functionally expressed with high efficiency and not $\alpha 1\delta\gamma 2\text{L}$ or $\delta\beta 1\gamma 2\text{L}$. $\alpha 1\beta 1\delta$ showed low but more detectable levels of expression of functional GABARs than $\alpha 1\delta\gamma 2\text{L}$ and $\delta\beta 1\gamma 2\text{L}$. Based on the larger amplitudes and greater efficiency of expression of $\alpha 1\beta 1\gamma 2\text{L}$ and $\alpha 1\beta 1\gamma 2\text{L}\delta$ GABAR channels compared to $\alpha 1\beta 1\delta$ GABAR channels, the former are more likely to make a significant contribution to GABA-gated Cl currents in these cells than the latter.

The acute phase of desensitization during the course of GABA application and the time course of recovery of GABA-evoked currents were slowed in the $\alpha 1\beta 1\gamma 2\text{L}\delta$ and $\alpha 1\beta 1\delta$ GABAR channels compared to the $\alpha 1\beta 1\gamma 2\text{L}$ GABAR channels. Though the drug application time of the recording system might affect the absolute values of rates of current rise and decay and estimates of τ_d and τ_r , the differences among the three different GABAR

Figure 9. Representative single-channel currents recorded from $\alpha 1\beta 1\gamma 2\text{L}$, $\alpha 1\beta 1\gamma 2\text{L}\delta$, and $\alpha 1\beta 1\delta$ GABARs. Excised outside-out membrane patches from LacZ+ L929 cells were held at -75 mV and 10 μM GABA was applied throughout the duration of the trace.

Table 2. Pharmacological profile of recombinant GABARs

	Enhancement by diazepam	Enhancement by pentobarbital	Block by zinc
$\alpha 1\beta 1\gamma 2L$	+	++	--
$\alpha 1\beta 1\gamma 2L\delta$	++	++	+
$\alpha 1\beta 1\delta$	--	++	++

Cells were held at -75 mV, and 5 sec pulses of either GABA alone or GABA with the relevant drug (diazepam, pentobarbital, or zinc) were applied and the enhancement by diazepam and pentobarbital and block by zinc compared. ++, strong indicated effect; +, moderate indicated effect; --, lack of indicated effect.

isoforms could not arise solely due to this, since the various GABAR isoforms were exposed to the same conditions of drug application. The $\alpha 1\beta 1\gamma 2L\delta$ and $\alpha 1\beta 1\gamma 2L$ GABAR channels showed a steep concentration–response (CR) curve and amplitudes that were 30–75 times larger than the $\alpha 1\beta 1\delta$ GABAR channels which also displayed a much shallower CR curve.

To prove that the δ subunit was functionally expressed in cells cotransfected with $\alpha 1$, $\beta 1$, and δ subunits and that the $\alpha 1\beta 1\delta$ GABARs in these cells were distinct from the dimeric $\alpha 1\beta 1$ GABARs, we examined the effect of the polyvalent cation lanthanum on $\alpha 1\beta 1$ and $\alpha 1\beta 1\delta$ GABAR isoforms. The $\alpha 1\beta 1$ GABAR currents were enhanced by lanthanum, but the $\alpha 1\beta 1\delta$ GABAR currents were neither enhanced nor inhibited by lanthanum. This suggests that coexpression of the δ subunit with $\alpha 1$ and $\beta 1$ subunits results in the formation of a functional GABAR with distinct pharmacological properties compared to $\alpha 1\beta 1$ GABARs and that the expression of the δ subunit with α and β subunits renders the $\alpha 1\beta 1\delta$ GABAR insensitive to potentiation by lanthanum. This also suggests that there are no significant levels of $\alpha 1\beta 1$ GABARs expressed in cells transfected with $\alpha 1$, $\beta 1$, and δ subunits since the subset of $\alpha 1\beta 1$ GABARs, if present, would have been enhanced by lanthanum contributing to increase in whole-cell current in the cells, which was not seen ($n = 3$). This supports our contention that $\alpha 1\beta 1\delta$ GABARs and not $\alpha 1\beta 1$ GABARs are likely to be the preferred final GABAR isoform in cells transfected with $\alpha 1$, $\beta 1$, and δ subunits.

Comparison of the single-channel properties of $\alpha 1\beta 1$ and $\alpha 1\beta 1\delta$ GABARs also confirms their distinct electrophysiological characteristics. Angelotti and Macdonald (1993) reported a main conductance state of 15 pS and mean open duration 2.3 msec for recombinant $\alpha 1\beta 1$ GABARs expressed in L929 cells, which is very different from the main conductance state of 22 pS and mean open duration of 400 msec for the $\alpha 1\beta 1\delta$ GABARs reported in this study.

Use of pharmacological agents revealed important similarities and differences among the various subunit combinations (Table 2). Currents mediated by all three subunit combinations were enhanced by pentobarbital as is characteristic of all GABARs regardless of subunit composition. However, they showed differential sensitivity to diazepam and the divalent cation, zinc (Zn^{2+}), whose actions are regulated by the subunit composition of GABARs. Diazepam enhanced the currents in GABARs containing the γ subunit ($\alpha 1\beta 1\gamma 2L\delta$ and $\alpha 1\beta 1\gamma 2L$ GABAR channels), but had no effect on those devoid of the γ subunit ($\alpha 1\beta 1\delta$ GABAR channels). As diazepam's effect of enhancing GABA-evoked currents was contingent on the presence of the γ subunit, this confirmed that the functional GABARs assembled in cells transfected with $\alpha 1\beta 1\gamma 2L\delta$ and $\alpha 1\beta 1\gamma 2L$ subunit combinations

contained the γ subunit. Zn^{2+} blocked the GABA-evoked $\alpha 1\beta 1\delta$ currents completely but did not block the $\alpha 1\beta 1\gamma 2L$ currents. This is consistent with previous findings that GABARs reconstituted in fibroblasts from combinations of α and β subunits are potently blocked by Zn^{2+} while the presence of γ subunit in any combination with α and β leads to the formation of GABARs that are almost insensitive to Zn^{2+} (Draguhn et al., 1990). Though the Zn^{2+} block of the $\alpha\beta$ GABAR isoform reported by Draguhn et al. was qualitatively similar to the Zn^{2+} block of GABA-activated currents reported in neuronal cells (Westbrook et al., 1987; Smart et al., 1990; Mayer and Vyklicky, 1989), Zn^{2+} blocked the $\alpha\beta$ isoform with much higher potency than the native GABAR channels in neurons. This difference in the potency of the Zn^{2+} block might reflect either a different pattern of GABAR subunit expression in neuronal membranes with the presence of an unidentified or novel subunit in neuronal membranes or a collection of several GABAR subtypes having different Zn^{2+} sensitivities. This suggests that the δ subunit modifies the Zn^{2+} insensitivity of GABARs containing the γ subunit and results in the functional expression of a new receptor subtype which shows susceptibility to block by Zn^{2+} . Interestingly, in the present study, Zn^{2+} blocked $\alpha 1\beta 1\gamma 2L\delta$ currents with lower potency than $\alpha 1\beta 1\delta$ currents, similar to that observed in neuronal membranes compared to $\alpha\beta$ isoforms (Westbrook et al., 1987; Mayer and Vyklicky, 1989; Smart et al., 1990). Since *in situ* hybridization studies have shown the colocalization of $\alpha 1$, $\beta 1$, $\gamma 2L$, and δ subunit mRNAs in the hippocampal dentate gyrus granule cells, the $\alpha 1\beta 1\gamma 2L\delta$ GABARs with intermediate sensitivity to block by Zn^{2+} reported in this study might be a physiologically relevant configuration of the native GABARs reported in neuronal membranes that show lower susceptibility to block by Zn^{2+} than the recombinant $\alpha\beta$ isoforms. The differential sensitivity to Zn^{2+} also provides a pharmacological tool to distinguish between $\alpha 1\beta 1\gamma 2L$ and $\alpha 1\beta 1\gamma 2L\delta$ GABAR isoforms since the former are insensitive to block by Zn^{2+} while the latter are susceptible to block by Zn^{2+} despite the presence of the γ subunit (as evidenced by diazepam-mediated enhancement of GABA-evoked currents). The two different mechanisms that could explain the difference in Zn^{2+} sensitivity are the formation of a novel GABAR isoform which shows lesser susceptibility to block by Zn^{2+} and the existence of a mosaic of different GABAR isoforms with different degrees of sensitivity to block by Zn^{2+} . We can not eliminate the possibility that dimers (like $\alpha 1\beta 1$) or oligomers (like $\alpha 1\beta 1\delta$ and $\alpha 1\beta 1\gamma 2L$) could also be formed besides the $\alpha 1\beta 1\delta$, $\alpha 1\beta 1\gamma 2L$, or $\alpha 1\beta 1\gamma 2L\delta$ GABAR isoforms in cells transfected with three and four different subunit combinations respectively. However, previous work of Angelotti and Macdonald (1993) and results reported here suggest that this is not very likely.

First, it has been reported earlier by Angelotti and Macdonald (1993) that $\alpha 1\beta 1\gamma 2S$ is likely to be the preferred final isoform of the GABAR in L929 cells transfected with $\alpha 1$, $\beta 1$, and $\gamma 2S$ subunits in a ratio of 1:1:1 or 1:2:1. This conclusion was based on the findings that no diazepam-insensitive currents were recorded from these cells, and that the currents showed a constant percentage enhancement by diazepam regardless of the ratio of the β to α or γ subunits. Detailed kinetic analysis of the single-channel characteristics also suggest that the $\alpha 1\beta 1\gamma 2S$ GABAR isoform was a preferred form of the GABAR relative to $\alpha 1\beta 1$ in cells transfected with $\alpha 1$, $\beta 1$, and $\gamma 2S$ subunits. Second, if the blocking effect of Zn^{2+} on $\alpha 1\beta 1\gamma 2L\delta$ GABARs was due just to two different populations of $\alpha 1\beta 1\delta$ and $\alpha 1\beta 1\gamma 2L$ GABARs,

it would be difficult to account for a 25% reduction in amplitude of the GABA-evoked currents (of average amplitude 667 ± 321 pA) solely by block of the poorly expressed $\alpha 1\beta 1\delta$ GABARs (GABA responsiveness of 18%, Table 1) with average amplitude of 41 ± 14 pA. To account for this, one would have to invoke the presence of a "novel" GABAR isoform with greater efficiency of expression, larger current amplitudes and sensitivity to block by zinc, since the $\alpha\beta\gamma$ GABARs have been shown to be insensitive to block by zinc (Draguhn et al., 1990; Verdoorn et al., 1990). Similarly, if the stimulatory effect of diazepam in cells transfected with $\alpha 1$, $\beta 1$, $\gamma 2L$, and δ subunits was just due to its action on a subset of $\alpha\beta\gamma$ GABARs, the percentage enhancement of GABA-evoked current by diazepam in these cells would be expected to be lower than or equal to that in cells transfected with $\alpha 1$, $\beta 1$, and $\gamma 2L$ subunits since the diazepam-insensitive subset of $\alpha 1\beta 1\delta$ GABARs would constitute part of the whole-cell current. On the contrary, 100 nM diazepam showed greater enhancement of $\alpha 1\beta 1\gamma 2L\delta$ GABAR currents ($60 \pm 21\%$, $n = 3$) than $\alpha 1\beta 1\gamma 2L$ GABAR currents (38 ± 19 , $n = 3$). Though this difference was not statistically significant, it is consistent with the report of Mertens et al. (1993) which demonstrated that diazepam-sensitive (and therefore γ -containing) GABARs isolated using δ -subunit specific antiserum show four- to fivefold greater affinity for diazepam than other diazepam-sensitive GABARs isolated using $\alpha 1$ or $\alpha 3$ -subunit specific antisera (Mertens et al., 1993). Finally, the single-channel characteristics of cells transfected with $\alpha\beta$, $\alpha\beta\delta$, $\alpha\beta\gamma$, and $\alpha\beta\gamma\delta$ subunits were distinct with main conductance levels of 15, 22, 30, and 33 pS, respectively. The mean open durations were 2.3, 400, 5, and 20 msec for $\alpha\beta$, $\alpha\beta\delta$, $\alpha\beta\gamma$, and $\alpha\beta\gamma\delta$ single channels, respectively. If there were "extra" oligomers present, one would expect to see different conductance levels or open times corresponding to these, for example, the dimeric receptors in patches pulled off cells transfected with $\alpha\beta\delta$ or $\alpha\beta\gamma$ subunits, which were not seen. Comparison of the characteristics of $\alpha 1\beta 1\gamma 2L$ and $\alpha 1\beta 1\gamma 2L\delta$ GABAR single-channel currents supports the possibility that the two form distinct GABAR isoforms since the average open channel duration of $\alpha 1\beta 1\gamma 2L\delta$ GABAR channels was at least four times longer than that of $\alpha 1\beta 1\gamma 2L$ GABAR channels (20 msec vs 5 msec). Though the presence of smaller duration $\alpha 1\beta 1\gamma 2L$ -like openings (5 msec) interspersed among the longer duration openings (20 msec) could not be ruled out, the majority of the current in cells transfected with $\alpha 1\beta 1\gamma 2L\delta$ subunits appeared to be carried via the novel $\alpha 1\beta 1\gamma 2L\delta$ GABAR channels with longer duration openings. However, more detailed kinetic analysis would be required to discriminate among different GABAR isoforms and determine if a final preferred form exists.

Therefore, these findings suggest that the cotransfection of L929 cells with $\alpha 1$, $\beta 1$, $\gamma 2L$, and δ subunit expression vectors leads to the formation of a novel functional GABAR isoform, $\alpha 1\beta 1\gamma 2L\delta$ GABAR, with distinct pharmacological and electrophysiological properties.

The main conductance levels of $\alpha 1\beta 1\gamma 2L$ and $\alpha 1\beta 1\gamma 2L\delta$ GABAR channels were comparable at 30 and 33 pS, respectively, while that of the $\alpha 1\beta 1\delta$ GABAR channels was lower at 22 pS. The latter also displayed much longer uninterrupted burst durations of 1–3 sec. The $\alpha 1\beta 1\gamma 2L$, $\alpha 1\beta 1\gamma 2L\delta$, and $\alpha 1\beta 1\delta$ GABAR channels with mean open times of 5, 20, and 400 msec showed increasing times for whole-cell GABA-evoked currents to recover to baseline after GABA application. Therefore, the single-channel properties of the three different GABAR isoforms are in agreement with the characteristics of their whole-cell currents.

Perspective on GABAR isoforms containing the δ subunit

In our studies, we did not observe functional expression of GABARs in L929 cells transfected with the δ subunit cDNA alone. Using high efficiency expression system of SF9 cells infected with baculoviruses containing cDNAs for various GABAR subunits, Hartnett et al. (1993) demonstrated that the subunit requirements for ligand binding are similar to those seen with GABARs in mammalian cells. No significant binding was seen with homomeric constructs containing α , β , γ , or δ subunits. In addition, constructs containing $\alpha\gamma$, $\beta\gamma$, $\alpha\delta$, or $\beta\delta$ also produced no significant binding. Minimal requirements for muscimol binding were at least one α and one β , similar to our findings in L929 cells (Angelotti et al., 1993). However, Shivers et al. (1989) have reported the functional expression of GABARs in human embryonic kidney cells (293 cells) transfected with the δ subunit alone. They reported whole-cell currents of approximately 50 pA and 150 pA in response to 10 and 100 μ M GABA, respectively. A possible explanation for this difference could be based on the different expression systems used in the two cases. There is some evidence for the presence of endogenous GABAR subunits, for example, $\beta 3$ in HEK293 cells (Kirkness and Fraser, 1993). Therefore, the functional expression of GABARs following transfection with a single subunit might reflect the presence or absence of other associated GABAR subunits crucial for the assembly of GABARs in the cell expression systems. However, it is not clear whether the level of endogenous proteins in these cells is sufficient to impact on the process of functional expression.

In situ hybridization studies (Laurie et al., 1992; Wisden et al., 1992) suggest the colocalization of $\alpha 1$, $\beta 1$, $\gamma 2L$, and δ subunit mRNAs in the granule cells of the dentate gyrus in the hippocampus. Using an antiserum specific for the δ subunit in immunoaffinity chromatography, the δ subunit was found to be associated with $\alpha 1$, $\beta 2/3$, and $\gamma 2$ subunits (along with the $\alpha 3$ subunit) (Mertens et al., 1993). Though experiments with $\beta 2/3$ have not been performed, based on the high degree of homology among the three β subunits (Ymer et al., 1989; also reviewed in Olsen and Tobin, 1990), the absence of $\beta 2$ subunit mRNA from the granule cells of the dentate gyrus (Wisden et al., 1992) and suggestions that the β subunit subtypes do not affect the determination of selectivity for benzodiazepine-site ligands (3H -Ro-15-1788; Ragan et al., 1993), the use of $\beta 1$ subunits in this study instead of $\beta 2/3$ appears reasonable.

Physiological implications of GABARs containing the δ subunit

Another characteristic property of GABARs, Zn^{2+} sensitivity, is regulated by the subunit composition. However, unlike the simple association of Zn^{2+} insensitivity with the γ subunit reported earlier (Draguhn et al., 1990), the coassembly of δ subunit with γ subunit renders the GABAR susceptible to block by Zn^{2+} . This suggests that the presence of the δ subunit creates a site to which Zn^{2+} can bind despite the presence of the γ subunit. This could cause a change in the structure of the channel from that in the absence of the δ subunit, which is consistent with the finding that the open channel duration of $\alpha 1\beta 1\gamma 2L\delta$ channels is much longer than that of the $\alpha 1\beta 1\gamma 2L$ channels. Therefore, the up- or downregulation of δ subunit-specific mRNA in particular regions of the brain could provide an efficient mechanism for modifying the Zn sensitivity of inhibitory synaptic transmission.

Shivers et al. (1989) observed prominent expression of δ subunit mRNA in neurons like the periglomerular cells of the ol-

factory bulb, the thalamocortical neurons and granule cells of the cerebellar cortex whose dendritic processes exist as glomeruli or rosettes and which function to limit the spread of excitatory impulses to neighboring cells, thus creating the surround-inhibition property. Insertion of GABARs having long channel open times and minimal desensitization, like the $\alpha 1\beta 1\gamma 2L\delta$ and $\alpha 1\beta 1\delta$ channels described here, into the more distally located synapses of glomeruli or rosettes may also be advantageous for efficient signal transmission.

Finally, specific regions of the brain like the dentate gyrus have recently become the focus of study in the etiology of seizures. Based on the results of *in situ* hybridization studies in the rat brain, the δ subunit mRNA is colocalized along with $\alpha 1$, $\beta 1$, and $\gamma 2L$ mRNAs in the hippocampal dentate gyrus granule cells, layer II/III of the neocortex and the pyriform cortex and could constitute GABARs of the $\alpha 1\beta 1\gamma 2L\delta$ and the $\alpha 1\beta 1\delta$ isoforms discussed in this paper. The reduced rate of acute desensitization and the slow recovery of GABA-evoked currents typical of these δ containing subunit combinations could generate tonic inhibition via long-lasting IPSPs and thus play a role in preventing seizures. By the same rationale, a reduction in the level of expression of the δ subunit mRNA in these regions could produce a reduction in dentate inhibition and thus could be associated with a reduced seizure threshold.

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