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Expression patterns of γ-aminobutyric acid type A receptor subunit mRNAs in primary cultures of granule neurons and astrocytes from neonatal rat cerebella

PATRIZIA BOVOLIN, MARIA RITA SANTI, GIULIA PUIA, ERMINIO COSTA, AND DENNIS GRAYSON*

Fidia–Georgetown Institute for the Neurosciences and Georgetown University, 3900 Reservoir Road, NW, Washington, DC 20007

Contributed by Erminio Costa, July 13, 1992

ABSTRACT **Using a competitive polymerase chain reaction assay, we have quantitated the absolute amounts of mRNA encoding 14 distinct subunits of the γ-aminobutyric acid type A ($GABA_A$) receptor in primary cultures of rat cerebellar granule neurons and cerebellar astrocytes. We found that the total amount of $GABA_A$ receptor subunit mRNA in astrocytes was 2 orders of magnitude lower than in neuronal cells. Furthermore, granule cell cultures expressed all 14 different $GABA_A$ subunit mRNAs, while the astroglial cultures contained detectable amounts of all the subunits expressed by granule cells except the α6 and the γ2L subunits. Of the α subunit family members, the α1, α5, and α6 mRNAs were prominent in granule cells, while the α1 and α2 mRNAs were abundant in astrocytes. Of the β receptor subunit mRNAs, the β1 and β3 mRNAs were abundantly expressed in both cultures. The γ2S and γ2L mRNAs constituted the great majority of γ subunit mRNAs in neurons, while the γ1 subunit mRNA was the most abundant γ subunit mRNA in astrocytes. When various allosteric modulators of $GABA_A$ receptors were tested electrophysiologically, methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM) was the only one to modulate chloride currents elicited by GABA in a significantly different manner in granule cells (negative modulation) compared with astrocytes (positive modulation). The latter effect was previously observed in transiently expressed recombinant $GABA_A$ receptors containing a γ1 instead of a γ2 subunit. Our quantitative mRNA results suggest that an important molecular determinant responsible for the DMCM-positive modulatory effect on astroglial native $GABA_A$ receptors is the presence of the γ1 subunit in the receptor assembly.**

The type A γ-aminobutyric acid ($GABA_A$) receptor is a heterooligomeric integral membrane protein that includes a Cl^- channel. Different combinations of subunits encoded by individual members of a large family of homologous genes (for reviews, see refs. 1–3) contribute to the extraordinary structural heterogeneity of this receptor. Its molecular diversity brings about a structurally dependent heterogeneity in the efficacy, potency, and direction of action of allosteric modulators that act on the extracellular domain of $GABA_A$ receptors, such as benzodiazepines (BZs), β-carbolines, and their congeners. A diversity of $GABA_A$ receptor subunit mRNAs are expressed at different developmental stages (4–7) and in different brain regions (2, 8), and *in vitro* the levels of expression of specific subunit mRNAs can be modified by changes in glutamate-mediated synaptic signaling (9).

$GABA_A$ receptors are present on the cytoplasmic membrane not only of neurons but also of cortical astrocytes (10). The glial $GABA_A$ receptor shares with its neuronal counterpart the susceptibility to the allosteric modulators that act on the extracellular receptor domain, but the direction of the modulation exerted by the β-carboline methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM) on GABA-gated Cl^- currents is negative in neurons (11, 12) and is positive in cortical astrocytes (13). To establish the molecular determinants that are responsible for this and other functional differences between $GABA_A$ receptors in neurons and astrocytes, we have compared the molecular and pharmacological features of different $GABA_A$ receptor subtypes in two primary culture systems—i.e., granule neurons (a morphologically highly monotypic culture) and astrocytes, both obtained from cerebella of 8-day-old rats.

Because of the extensive heterogeneity of $GABA_A$ receptors, available biochemical and pharmacological tools are not sufficient to describe or infer the stoichiometry, the subunit composition of each $GABA_A$ receptor subtype, or the functional and pharmacological properties of one native receptor subtype versus another. It was our intent to see whether information relevant to the subunit stoichiometry of $GABA_A$ receptors might be obtained by quantitating the amounts of mRNAs encoding specific $GABA_A$ receptor subunits. Taking this approach, we have adapted a competitive polymerase chain reaction (PCR) assay (14–16) that makes use of subunit-specific amplification primers and internal standards (4, 17) for the quantitation of both high- and low-abundance subunit mRNAs. This highly sensitive method has allowed us to compare the absolute amounts of the mRNAs encoding 14 different $GABA_A$ receptor subunits expressed by cerebellar granule cells and astrocytes in primary culture. We also tested the allosteric modulatory activity of diazepam, DMCM, *N,N*-6-trimethyl-2-(4-methylphenyl)imidazo[1,2-*a*]pyridine-3-acetamide hemitartrate (zolpidem), and 7-chloro-1,3-dihydro-1-methyl-5-(4-chlorophenyl)-2*H*-[1,4]-benzodiazepin-2-one (4′-chlorodiazepam or Ro5-4864) on GABA-gated Cl^- currents in these cells. Using as a guideline the allosteric modulatory activity of these drugs, we found it impossible to reconcile the stoichiometry indicated by the amounts of the subunit mRNAs expressed by granule cells with any possible subunit stoichiometry suggested by the electrophysiology. One possible explanation is that specific signals determine the assembly of few receptor subtypes from a larger menu of subunit mRNAs that is available in the neuronal and astrocyte cultures. The following alternatives should also be considered: (*i*) marked differences exist in the translational rates of the mRNAs encoding different $GABA_A$ receptor subunits; (*ii*) the degree of maturation among granule neurons is not uniform and some express immature forms

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Abbreviations: GABA, γ-aminobutyric acid; $GABA_A$ receptor, type A GABA receptor; BZ, benzodiazepine; DMCM, methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate; zolpidem, *N,N*-6-trimethyl-2-(4-methylphenyl)imidazo[1,2-*a*]pyridine-3-acetamide hemitartrate; 4′-chlorodiazepam, 7-chloro-1,3-dihydro-1-methyl-5-(4-chlorophenyl)-2*H*-[1,4]-benzodiazepin-2-one; cRNA, complementary RNA.

*To whom reprint requests should be addressed.

of receptors that include multiple α subunits; (*iii*) granule neurons in culture contain subpopulations of cells which, despite their morphological similarity, differ in the qualitative and quantitative expression of $GABA_A$ receptor subunits.

MATERIALS AND METHODS

Cell Culture. Primary cultures of cerebellar neurons enriched in granule cells were prepared from cerebella of 8-day-old rats (18–20). Cells were plated (2.5×10^7 cells per dish) onto 100-mm Nunc dishes coated with poly(D-lysine) (Sigma) at 10 μg/ml and were cultured in basal Eagle's medium (GIBCO) containing 10% heat-inactivated fetal bovine serum (GIBCO), 2 mM glutamine, 25 mM KCl, and gentamicin (Sigma) at 100 μg/ml. Cytosine arabinofuranonucleoside (10 mM) (Sigma) was added 18 hr after seeding to inhibit the proliferation of nonneuronal cells.

Primary astrocyte cultures were prepared from cerebella of 8-day-old rats by following the same procedure used to dissociate the neuronal cells except that the astrocytes were seeded onto uncoated 100-mm dishes. To minimize culture contamination with fibroblasts (21), the glial cells were grown in D-valine-substituted minimal essential medium (GIBCO) supplemented with 2 mM glutamine, gentamicin at 100 μg/ml, and 10% heat-inactivated fetal bovine serum. Double immunofluorescence staining with antibodies specific for glial fibrillary acidic protein (GFAP) and A2B5 showed that about 90% of the cells in the astroglial cultures were GFAP-positive (type 1 and type 2 astrocytes) and that of this percentage, 10–15% were A2B5-positive type 2 astrocytes (data not shown).

Neurons were harvested in 5 M guanidine isothiocyanate (BRL) on the 8th day *in vitro*, while astrocytes were harvested after reaching confluence (after 11–13 days *in vitro*). Total RNA from both was isolated by extraction with guanidine isothiocyanate and ultracentrifugation in CsCl as previously described (22). The yield of total RNA was determined by measuring the absorbance of an aliquot of the ethanol-precipitated stock at 260 and 280 nm. DNA was extracted from both neuronal and glial cultures by using a DNA Extraction Kit (Stratagene), while the amount of DNA was determined by using the fluorescent dye Hoechst 33258 and the Hoefer model TKO 100 minifluorometer.

Competitive PCR Amplification. To quantitate the amount of each $GABA_A$ subunit mRNA expressed, we used competitive PCR (14–16) with subunit-specific primer pairs and internal standards that were generated for each $GABA_A$ receptor subunit mRNA to be amplified (4, 17). The amplification primers were derived from the nucleotide sequence encoding the intracellular loop domain of the respective receptor subunits because they correspond to unique regions of each subunit cDNA. Internal standards containing targeted restriction enzyme cleavage sites (*Bgl* II) were synthesized by overlap extension PCR (23) and cloned in pGEM-1 (Promega). The α1, α5, γ2L, and γ2S internal standards employed in the present work were designed and synthesized as described previously (4). Internal standards corresponding to the α2, α3, α4, α6, β1, β2, β3, γ1, γ3, and δ subunit mRNAs were similarly constructed and cloned (see ref. 17).

Complementary RNA (cRNA) was synthesized *in vitro* from each internal standard after linearization with *Sph* I by using T7 RNA polymerase as described by the supplier (Promega) for nonradioactive syntheses. For the competitive PCR assay, various amounts of cRNA prepared from the appropriate standard template were added to a constant amount of total RNA (1 μg for glial cells and 250 ng for neurons). The RNA/cRNA mixtures were reverse transcribed with 200 units of Moloney murine leukemia virus reverse transcriptase (BRL) with 2.5 μM random hexamer primers (Pharmacia) in a final volume of 20 μl as recommended by the supplier (BRL). Aliquots of the ethanol-precipitated cDNA containing 50–100% of the reverse-transcribed material were amplified with Hot Tub DNA polymerase (Amersham) in a thermal cycler as described in detail elsewhere (4). Amplification products arising from both the mRNA and cRNA templates were distinguished by digestion with *Bgl* II and separation by agarose gel electrophoresis as described (4).

Electrophysiology. Cultures of glial cells were studied with the single-electrode voltage-clamp technique in the whole-cell configuration (24) on the stage of an inverted microscope (Zeiss IM-35) at room temperature. Recordings from the granule neurons were made on the 8th day *in vitro* and within 8–12 days of plating of the cerebellar astrocytes. The recording pipette contained 145 mM CsCl, 1 mM $MgCl_2$, 11 nM EGTA, and 10 mM Hepes/CsOH, pH 7.2. Cells were bathed in 145 mM NaCl/5 mM KCl/2 mM $CaCl_2$/5 mM Hepes/NaOH, pH 7.4. GABA (0.5 M in H_2O adjusted to pH 4 with HCl) was applied by iontophoresis (25–50 nA) with 30-msec pulses of positive current. BZs were a gift from Hoffmann–La Roche, DMCM was from Ferrosan (Copenhagen), and zolpidem was from Syntelabo (Paris). All drugs were dissolved in bath solution containing dimethyl sulfoxide at a maximal final concentration of 0.1%. Application of bath medium alone failed to modify the responses to GABA. Drugs were applied by pressure [2–4 psi (14–28 kPa)] for 5 sec between two GABA pulses every 10 sec in the proximity of the cell body with micropipettes 5–10 μm in diameter. The maximal Cl^- current measured from each cell was larger (>1 nA) than the test response of 150–200 pA we used, indicating that the percentages of potentiation observed were far below the maximal efficacy of the system. Current traces were recorded by a patch clamp amplifier (EPC-7; List Electronics, Darmstadt, F.R.G.), filtered at 1500 Hz (eight-pole low-pass Bessel; Frequency Devices, Haverhill, MA), and recorded on a chart recorder (model 2600S; Gould, Cleveland, OH) for off-line analysis.

RESULTS

$GABA_A$ Receptor Subunit mRNA Content in Cerebellar Granule and Astroglial Primary Cultures Assayed by Competitive PCR. To measure absolute amounts of each $GABA_A$ receptor subunit mRNA, we have adapted a competitive-PCR-derived assay. For each receptor subunit mRNA determination, differing amounts of internal standard cRNA were added to total RNA, and the mixtures were reverse transcribed and PCR amplified (4). Each subunit mRNA was measured in three or four different culture preparations and is expressed as the mean ± SEM of attomoles (amol = 10^{-18} mol) of specific subunit mRNA per μg of total RNA (Table 1). To establish whether the RNA per cell content was the same for both glial and neuronal cultures, we measured the RNA and DNA contents in the culture dishes. The RNA-to-DNA ratio was 0.66 for granule cells and 0.64 for the confluent astrocytes, indicating that these two cell populations express comparable amounts of RNA per cell. We also determined the lower limits of quantitation of our competitive PCR assay. For this purpose, 1 μg of total RNA extracted from granule cells was serially diluted (to 1:15,000 or 67 fg) and the dilutions were used to measure corresponding amounts of α6 receptor subunit mRNA. The absolute quantities of α6 mRNA anticipated to be present in each dilution were compared with those determined experimentally. We found a good agreement between the calculated and experimentally determined values down to 0.05 amol of α6 mRNA (data not shown). Although we can detect a number of molecules lower than this, the quantitation below this number is accompanied by an unacceptable high error.

Table 1. $GABA_A$ receptor subunit mRNA contents

Subunit	mRNA, amol/μg of total RNA	
	Granule cells	Glial cells
$\alpha 1$	520 ± 64	3.5 ± 0.52
$\alpha 2$	7.1 ± 0.97	1.3 ± 0.38
$\alpha 3$	7.6 ± 0.48	0.12 ± 0.065
$\alpha 4$	37 ± 8.5	0.20 ± 0.044
$\alpha 5$	130 ± 41	0.34 ± 0.23
$\alpha 6$	250 ± 44	ND
$\beta 1$	120 ± 14	2.9 ± 0.86
$\beta 2$	26 ± 7.9	0.10 ± 0.044
$\beta 3$	130 ± 24	1.9 ± 0.19
$\gamma 1$	1.9 ± 0.12	0.55 ± 0.21
$\gamma 2L$	25 ± 4.3	ND
$\gamma 2S$	120 ± 38	0.11 ± 0.025
$\gamma 3$	1.3 ± 0.29	0.17 ± 0.026
δ	17 ± 3.1	0.088 ± 0.025

Data for granule cells and glia are presented in attomoles (10^{-18} mol)/μg of total RNA. Values represent the mean (±SEM) of three to five different competitive PCR experiments, each performed from a different cell culture preparation. ND, not detectable after 34 PCR cycles and starting with 2 μg of total RNA.

The $GABA_A$ receptor subunit mRNA content of granule cell cultures is 120-fold higher than that of the astroglial cultures. In the latter, several subunit mRNAs were either below the limit of detection ($\alpha 6$, $\gamma 2L$) or were present at very low levels ($\alpha 3$, $\beta 2$, $\gamma 2S$, δ). In granule neurons the predominant subunits (68% of total) are those encoding the α family, while in astrocytes the mRNA content corresponding to the α (48% of total) and β (43% of total) subunit families are comparable. Moreover, expression of the $\alpha 1$ and $\alpha 2$ mRNAs predominates in astrocytes, whereas the granule neurons preferentially express mRNAs encoding the $\alpha 5$ and $\alpha 6$ subunits in addition to the $\alpha 1$, which is the most abundant. Among the β subunit mRNAs, the $\beta 2$ mRNA content is the lowest in both cultures, while the mRNAs for $\beta 1$ and $\beta 3$ are expressed at comparable levels (the $\beta 1$ mRNA is slightly more abundant in astrocytes). mRNAs encoding the γ and δ subunits represent a smaller fraction of the total subunit mRNA content in both cultures, with the difference being that in granule neurons the predominant subunit mRNA of this class is the $\gamma 2S$. In the glial cultures, the predominant subunit of this class is the $\gamma 1$ (approximately 66% of the γ subunits plus δ subunit).

Allosteric Modulation of GABA-Activated Cl^- Currents in Cultured Granule Neurons and Astrocytes. Using the patch-clamp technique in the whole-cell configuration, we studied the allosteric modulation of GABA-evoked Cl^- currents in the granule cell cultures and astrocyte cultures employed for the quantitative assay of $GABA_A$ receptor subunit mRNAs. Because of the technical difficulties encountered in recording from the more flat, type 1 astrocytes, our experiments were performed mainly on round cells, immunocytochemically identified as type 2 astrocytes. In fact, the type 1 cells respond very slowly to iontophoretically applied GABA, and the GABA response desensitized rapidly (data not shown). The resting potential of the round cells was −64 ± 10 mV (mean ± SEM), and 90% of the cells recorded from were sensitive to GABA and in all of them GABA-gated Cl^- currents could be affected by the allosteric modulators under study (total number of cells recorded from was 30). In astroglial cultures, both diazepam (10 μM) and zolpidem (10 μM) positively modulated the GABA-gated Cl^- currents by nearly 70% (Fig. 1). The β-carboline derivative DMCM (10 μM) positively modulated the GABA-activated Cl^- currents by only 20% (Fig. 1), while 4′-chlorodiazepam (10 μM) negatively modulated the GABA-gated Cl^- currents by 60% (Fig. 1). In primary cultures of granule neurons at 8 days in culture GABA-activated Cl^- current was potentiated by diazepam by 70% ± 25% (mean ± SEM, n = 7) and by zolpidem (n = 4) by 60% ± 11%, while 4′-chlorodiazepam and DMCM decreased the Cl^- current elicited by GABA by 45% ± 10% (n = 4) and 30% ± 7% (n = 4), respectively.

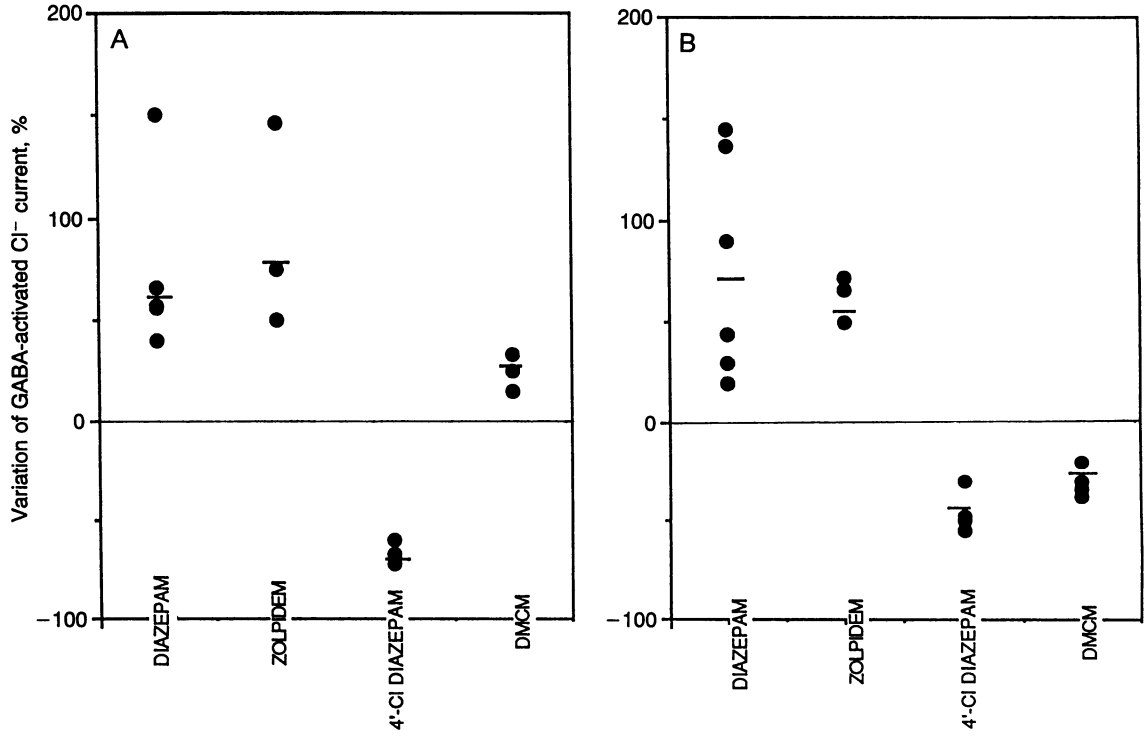

FIG. 1. Modulation of GABA-elicited Cl^- currents in primary cultures of astrocytes (*A*) and granule cells (*B*). Each dot represents the percent modulation (positive or negative) of Cl^- current obtained from a single cell after the application of the drug (diazepam, zolpidem, 4′-chlorodiazepam, DMCM). Horizontal bars correspond to the mean value of all the experiments.

DISCUSSION

Our data show that morphologically monotypic (90%) primary cultures of cerebellar granule neurons express mRNAs encoding 14 different $GABA_A$ receptor subunits. This great variety in the expression of receptor subunits is maintained at later stages in culture (4) and in the granule cells of adult cerebella (25), although the relative amounts of some subunit mRNAs ($\alpha5$, $\beta1$, $\beta2$, δ) differ substantially. With so many subunits available, assuming a pentameric quaternary structure, the expression of thousands of stoichiometrically different subunit assemblies is theoretically possible. However, the number of subunit combinations of native receptors is probably smaller than the theoretical combinatorial possibilities. Here we clearly show that the mRNAs encoding different $GABA_A$ receptor subunits are unequally expressed (Table 1), suggesting that only a limited number of subunit combinations are used by granule cells. Furthermore, recent immunoprecipitation studies with the limited number of subunit-specific antibodies available suggest that most receptor combinations contain only one type of α subunit, and not a mixture of different αs (26, 27). If each receptor expressed by cerebellar granule neurons is mainly made by only one type of α subunit, there would be three major receptor subtypes present at 8 days in culture, one containing an $\alpha1$ subunit, one an $\alpha6$, and one an $\alpha5$, thereby drastically restricting the number of possible receptor stoichiometries.

Our quantitative mRNA results also indicate that the amount of the total α subunit mRNAs is 3 times higher than the β and 6 times higher than the γ. Even assuming that each receptor assembly contains multiple α subunits (2 or 3), and fewer β subunits (1 or 2), on the basis of mRNA abundances a substantial number of subunit assemblies should contain no γ subunits. This would have important functional consequences, since allosteric modulation of GABA-gated Cl^- currents requires the presence of a γ subunit in recombinant $GABA_A$ receptors (28). We tested by electrophysiology whether the functional requisites of the $GABA_A$ receptors expressed by granule neurons corresponded to those of $\gamma2S$- and $\gamma2L$-containing (the most expressed mRNAs of the γ family) recombinant subunit assemblies. To our surprise, we found that the Cl^- current activated by GABA was positively (diazepam and zolpidem) and negatively (DMCM, 4′-chlorodiazepam) modified by allosteric modulators in 90% of the granule cells that we tested. The simplest explanation would be that most subunit assemblies contain a $\gamma2S$ or $\gamma2L$ subunit, suggesting that mRNA abundances are not sufficient to predict receptor subunit stoichiometry in granule neurons. Variations in the translational rates of the mRNAs encoding different receptor subunits and/or subunit selectivity in receptor assembly may play a dominant role in determining the stoichiometry of $GABA_A$ receptors functionally expressed at cell surfaces. The electrophysiological tests also showed that the degree of potentiation of GABA action by diazepam varies considerably from cell to cell (Fig. 1). Because the whole cell patch-clamp recording measures the total current flowing through all $GABA_A$ receptors in one cell, it is possible that a comparably less effective modulation is due to the expression of a mixture of BZ-sensitive (i.e., containing $\alpha1$, $\alpha5$, and $\gamma2$) and BZ-insensitive (i.e., containing $\alpha6$ but no γ subunits) $GABA_A$ receptors. This could mean that different sets of $GABA_A$ receptor subunits are expressed by subpopulations of granule cells in culture. Since different receptor subunit stoichiometries are characterized by a structurally dependent (type of α, presence or absence of a γ) degree of modulation by BZs, each granule cell would have distinct functional attributes which depend on the different proportions of each $GABA_A$ receptor subtype present.

Are there specific regulatory factors for the functional expression of a given subunit combination versus another? Our results are in favor of the hypothesis that a full menu of receptor subunit mRNAs is readily available in each granule neuron, and it might even be qualitatively and/or quantitatively different from neuron to neuron at any particular day in culture. The steady-state level of subunit mRNAs in each neuron is very likely regulated by a combination of chemical signals coming from the environment, such as the release of glutamate (9), regulatory peptides, steroid hormones, etc. These chemical signals could act through immediate early genes, which in turn would activate distinct transcriptional programs and different mRNA degradation rates for each subunit mRNA. In addition to this, certain subunit mRNAs (i.e., $\alpha1$) could be produced in excess and the expression of the corresponding protein could be regulated at the translational level (i.e., only a limited number of $\alpha1$ mRNAs is translated into protein). Furthermore, specific subunit protein could accumulate in a cytoplasmic compartment without being immediately incorporated into a functional receptor. A posttranscriptional regulation of receptor subunit assembling (making use of already transcribed, available mRNAs) could represent a mechanism of fast cellular plasticity in response to incoming signals. Some receptor stoichiometries (i.e., containing multiple α subunits) could be typically assembled by neurons which are still functionally immature due to their incomplete or delayed synaptic connectivity. To address these issues, studies using a full series of subunit-specific antibodies will be necessary.

The interpretation of the mRNA data in the astroglial cultures seems to be easier. The total amount of α and β subunit mRNAs in astroglial cells is approximately the same. The most abundant α subunits are $\alpha1$ and $\alpha2$, which suggests that at least two different receptor subtypes may be present in this culture. The most significant difference between the $GABA_A$ receptors present in granule neuron and astroglial cultures is the differential expression of the γ subunits. That is, the $\gamma2S$ + $\gamma2L$ constitute 98% of the total γ subunits present in neurons, while the $\gamma1$ subunit mRNA represents 67% (with the $\gamma3$ and the $\gamma2S$ making up 20% and 13%, respectively) of the γ subunits in the glial cultures. That there are functional consequences of this stoichiometry of mRNAs expressed by glia is supported by the electrophysiological data. The majority of the tested astrocytes show a low BZ potentiation, as can be expected for receptors not containing $\gamma2$ subunits; the exceptions (high potentiation) may represent astroglial cells containing subunit assemblies with $\gamma2S$ subunits in addition to receptors containing $\gamma1$ and $\gamma3$ subunits. As mentioned in the introduction, DMCM negatively modulates $GABA_A$ receptors in granule cells, and it seems to do so also in most neuronal cell types, as for instance cortical neurons (29, 30). Conversely, we observed that the effect of DMCM on $GABA_A$ receptors of cerebellar astrocytes is a moderate, but consistent, positive modulation; a similar result has been described for cortical astrocytes (13). It has also been reported that DMCM negatively modulates GABA currents in recombinant receptors including either $\gamma2$ or $\gamma3$ subunits, while it acts as a positive modulator on receptor assemblies containing $\gamma1$ (29, 31). The results of our quantitative mRNA analysis suggest that one important molecular determinant which specifies this functional difference between glial and neuronal $GABA_A$ receptors is the presence of a $\gamma1$ instead of a $\gamma2$ subunit in the receptor assembly. 4′-Chlorodiazepam, a flumazenil-resistant negative modulator of GABA action (11), does not revert its action in cultured astrocytes. In fact, the direction of 4′-chlorodiazepam modulation is independent of the type of γ or other subunit present in the receptor assembly (29). Since $GABA_A$ receptors expressed in cortical astrocytes display a pharmacology (13) similar to that expressed in cerebellar astrocytes examined in the present study, we suggest that the molecular structure that is responsible for these functional properties

could represent a general feature of $GABA_A$ receptors present in astrocytes from different brain regions.

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