Quantitative Characterization of α6 and α1α6 Subunit-containing Native γ-Aminobutyric AcidA Receptors of Adult Rat Cerebellum Demonstrates Two α Subunits per Receptor Oligomer*

(Received for publication, May 23, 1995, and in revised form, July, 7, 1995)

Simon Pollard, Christopher L. Thompson, and F. Anne Stephenson†

From the Department of Pharmaceutical Chemistry, School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX, United Kingdom

γ-Aminobutyric acidA (GABA)A receptors were purified from adult rat cerebella by anti-α6(1-16 Cys) antibody affinity chromatography. Immunoblots of the α6 subunit-containing receptors showed the copurification of the α1, β2/3, γ2, δ but not α2 and α3 GABA receptors polypeptides. Further fractionation of this receptor subpopulation by anti-GABA receptor subunit α6(1-16 Cys) and anti-α1(413-429) antibody affinity columns in series substantiated the coassociation of the α1 and α6 polypeptides. The percentage of coexistence of the two subunits was determined by quantitative immunoblotting, which found that 41 ± 12% of α6 subunit immunoreactivity is associated with the α1 subunit. The ratios of the α1α6 subunits in the double purified receptor preparations was found to be 1:1, thus determining directly for the first time subunit ratios within native GABA receptors. The benzodiazepine pharmacology of the α1α6 subunit-containing receptors was shown to be predominantly benzodiazepine-insensitive by quantitative immunoprecipitation assays. These results are the first direct quantitative studies of subunit ratios within a population of native GABA receptors.

The GABA receptors of mammalian brain are fast-acting ligand-gated chloride ion channels. Multiple genes encoding GABA receptor subunits have been identified by molecular cloning. These are classified on the basis of their respective amino acid sequence similarities into five subunit types. Thus known mammalian GABA receptor subunits are the α1-6, β1-3, γ1-3, δ, and ρ1-2 subunits, comprising 15 identified to date (for review, see McDonald and Olsen (1994)). Different combinations of these subunits are thought to assemble, probably in pentameric combinations, in vivo to form functional GABA receptors. The polypeptide complement of any one native GABA receptor has not been elucidated. However, the biochemical and pharmacological properties of cloned receptors suggest that most probably consist of an αβγ combination (Olsen and McDonald, 1994; Stephenson, 1995). A subunit stoichiometry (αβγ) was found for a defined expressed GABA receptor (Backus et al., 1993). For native receptors, a five-fold axis of symmetry was revealed by negative stain electron microscopy thus providing the first evidence for a pentameric quaternary structure in vivo (Nayeem et al., 1994). Furthermore, Khan et al. (1994) recently deduced by immunoprecipitation studies that a GABA receptor subpopulation in the cerebellum consisted of α1α6β2γ2β2/3 subunits where γ2 and γ2 are splice variants of the γ2 subunit.

We have adopted the approach of the determination of native GABA receptor subunit complements by the purification of subsets of receptors by immunobioaffinity chromatography using subunit-specific antibodies (e.g. Duggan et al. (1991) and Pollard et al. (1993)). We have focused primarily on the α subunit complements of native receptors. We found, in agreement with several other groups, that the majority of native GABA receptors contain a single α subunit variant (e.g. Duggan et al. (1991), McKernan et al. (1991), and Benke et al. (1991)). However, we identified, by the use of different specificity antibody affinity columns in series, minor receptor populations that were heterogeneous with respect to their α subunit complement e.g. α1α2, α1α3, and α2α3 subunit-containing receptors (Duggan et al., 1991; Pollard et al., 1993). Thus, we proposed the assembly of at least two α subunits per receptor oligomer. Several other groups have since reported the copurification or coimmunoprecipitation of other α subunit variants (e.g. Mertens et al. (1993) and Kern and Sieghart (1994); summarized by Stephenson (1995)).

We have recently concentrated efforts on the GABA receptor α6 subunit-containing receptors. This is because this subunit is uniquely expressed in adult rat brain in a single cell type, the cerebellar granule cell (Luddens et al., 1990; Thompson et al., 1992). Furthermore, cloned α6β2γX receptors (and also α6βγX) receptors have a distinct benzodiazepine pharmacology in that they have high affinity for the partial inverse agonist Ro 15-4513 but very low affinity for the classical allosteric benzodiazepine regulators such as diazepam (Luddens et al., 1990). This pharmacological profile corresponds to the previously described diazepam-insensitive site (abbreviated in this paper to the benzodiazepine-insensitive site, BZ-IS) (Sieghart et al., 1991). We previously reported the purification of calf cerebellar α6 subunit-containing GABA receptors, but low yields in the isolation procedure precluded their detailed characterization (Pollard et al., 1993). The isolation efficiency has been improved by using adult rat cerebellum as starting material, thus permitting further analysis, including for the first time quantitative measurements of immunoreactivity. We report these results in this paper.

EXPERIMENTAL PROCEDURES

Materials

[3H]Ro 15-4513 (24:3 21285]
Ci/mmol) was from Du Pont (UK) Ltd. (Stevenage, Hertfordshire, UK). Fluoritrazepam and Ro 15-4513 were from Research Biochemicals Inc. (Natick, MA). Peptides, anti-peptide antibodies, and characterization of their specificities were as described by Thompson et al. (1992) and Pollard et al. (1993); details of the anti-GABA$_A$ receptor $\delta$ subunit antibodies are given below. The monoclonal antibody bd-17, which recognizes both GABA$_A$ receptor $\beta$2 and $\beta$3 subunits (Ewert et al., 1990), was from Boehringer Mannheim (Lewes, East Sussex, UK). All other materials were from commercial sources.

Methods

Production of GABA$_A$ Receptor $\delta$ Subunit Antibodies—The peptides $\delta$(320–337 Cys), DYRKRRKAVKVTKPRAEC, and $\delta$(2–12), PHGGRAMNDIC, were coupled via the C-terminal cysteine to keyhole limpet hemocyanin. Polyclonal antibodies were raised in rabbits and were affinity-purified by the respective peptide affinity resins all as described previously (Stephenson and Duggan, 1991). Both specificity anti-$\delta$ subunit antibodies recognized a M$_r$ 57,000 $\pm$ 500 immunoreactive band in addition to a protein with M$_r$ 64,000. The higher molecular weight species has been observed with other cysteine-coupled peptides with completely different amino acid sequences; it is a nonspecific, non-$\delta$ subunit protein.

Preparation of Membrane-bound, Detergent-solubilized, and Detergent-treated Membrane Fractions from Rat and Calf Brain—Membranes and Na$^+$ deoxycholate extracts of rat and calf cerebellum were prepared as described previously (Duggan and Stephenson, 1990). The pellet obtained after Na$^+$ deoxycholate solubilization was rehomogenized with 10 mM HEPES, pH 7.4, containing 1 mM EDTA, 1 mM benzamidine, centrifuged at 20,000 $\times$ g for 30 min at 4°C, and the pellet retained. This was then resuspended as above and termed the detergent-treated membranes.

Immunoinfinity Purification of GABA$_A$ Receptors—Immunoinfinity purification of GABA$_A$ receptors from Na$^+$ deoxycholate extracts of adult rat cerebellum was carried out using a rabbit anti-6(1–16 Cys) Fab antibody fragment affinity column, a sheep anti-1(413–429) whole antibody affinity column, or the anti-6(1–16 Cys) Fab and anti-1(413–429) antibody affinity columns in series as described previously, except that in the double immuninfinity column purification experiments, 0.05% (w/v) bovine serum albumin was added to the single GABA$_A$ receptor $\alpha$6 subunit-purified material (Duggan et al., 1991; Pollard et al., 1993). Purified receptor subpopulations were analyzed by both immunoblotting and radioligand binding assays.

Polyacrylamide Gel Electrophoresis and Immunoblotting—Polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions in 10% polyacrylamide slab gels, the chloroform/methanol method for protein precipitation, and the ECL Western blotting system for detection using the Molecular Dynamics personal densitometer.

Radioligand Binding and Immunoprecipitation Assays—Radioligand binding and immunoprecipitation assays were both carried out as described previously using the polyethyleneimine assay for the measurement of specific ligand binding activities (Stephenson and Duggan, 1990). For the measurement of $[^3H]$Ro 15-4513 binding activity, total binding was determined using 10 $\mu$M Ro 15-4513 to define nonspecific binding. For the measurement of benzodiazepine-sensitive (BZ-S) $[^3H]$Ro 15-4513 binding activity, 10 $\mu$M flunitrazepam was used in the binding assay. BZ-IS $[^3H]$Ro 15-4513 binding activity was thus calculated as the difference between total and the benzodiazepine-sensitive (BZ-S) binding activity.

RESULTS

We have previously reported the purification of $\alpha$6 subunit-containing GABA$_A$ receptors from calf cerebellum by anti-$\alpha$6(1–16 Cys) Fab antibody affinity chromatography (Pollard et al., 1993). In that study we reported the copurification of the $\alpha$6 and $\alpha$1 subunit immunoreactivities. However, because of the low yield in the isolation procedure, we were unable to characterize in detail the coassociation of $\alpha$6 and $\alpha$1 subunit immunoreactivities by the use of different specificity antibody columns in series, as has been employed for other GABA$_A$ receptor subpopulations (cf. Duggan et al., 1991; Pollard et al., 1993). Furthermore, there were no detectable radioligand binding activities in the purified receptor preparations, thus precluding the characterization of the pharmacological properties of native GABA$_A$ receptor $\alpha$6 subunit-containing receptors. An investigation of the efficiency of the purification procedure showed that for the standard conditions of receptor solubilization using 0.5% (w/v) Na$^+$ deoxycholate and 150 mM KCl, the majority of $\alpha$6 subunit immunoreactivity remained in the calf cerebellar detergent-treated membranes (Fig. 1). This was in contrast to the efficiency of solubilization under the same conditions from adult rat cerebellum, where at least 50% of the $\alpha$6 subunit immunoreactivity was found in the solubilized preparation (Fig. 1). The comparative results for the extraction of BZ-S and BZ-IS $[^3H]$Ro 15-4513 binding sites from rat cerebellum are shown in Table I. There was no appreciable difference in the efficiency of detergent solubilization between the two pharmacological classes of receptor. Furthermore, the percentage efficiency of solubilization of binding sites agreed with that found for $\alpha$6 subunit immunoreactivity (Table I and Fig. 1). This is in contrast to Uusi-Oukari (1992), who showed that BZ-S sites were preferentially solubilized from pig cerebellum. Although this differential sensitivity to detergent extraction appears to be species-dependent, it is important also to make the point that it may reflect differences between receptor subtypes in their subcellular compartmentalization and/or association with cytoskeletal elements particularly at less accessible sites such as synapses. For the results herein and indeed for all other papers addressing native GABA$_A$ receptor subunit complements by biochemical approaches, the assumption is made that the solubilized preparation is representative of the entire functional, $\alpha$6 subunit-containing GABA$_A$ receptor population.

Purification and Characterization of $\alpha$6 Subunit-containing GABA$_A$ Receptors from Adult Rat Cerebellum—The alternative use of rat cerebellum as the source of $\alpha$6 subunit-containing GABA$_A$ receptors with the increased efficiency of solubilization permitted their detailed biochemical characterization. Table I shows the $[^3H]$Ro 15-4513 binding results of a typical purification of GABA$_A$ receptors from adult rat cerebellum by anti-$\alpha$6(1–16 Cys) Fab immunoaffinity chromatography. The pH 11.5 elution profile of the total $[^3H]$Ro 15-4513 binding activity from the anti-$\alpha$6 subunit antibody column was concomitant with the M$_r$ 57,000 $\alpha$6 immunoreactive band (results not shown, but see also Fig. 3). For total $[^3H]$Ro 15-4513 binding...
activity, it can be seen that 18% of the sites applied are retained by the anti-α6(1–16 Cys) Fab antibody column. There is a recovery of 4% of the total [3H]Ro 15-4513 sites following pH 11.5 elution. This efficiency is the same order of magnitude found using different specificity GABA_A receptor antibody columns, e.g., for α1 and α2 (Duggan et al., 1991). When the pharmacology of the total [3H]Ro 15-4513 binding activity bound to anti-α6(1–16 Cys) Fab antibody column was subfractionated into the BZ-S and BZ-IS subunits, typical values were found 75% of the BZ-IS subunits when compared to 7% of the BZ-S subunits (Table II). Mean values obtained for the retention of BZ-IS and BZ-S were 3% and 2% respectively (n = 4). From Table III, it can be seen that the percentage of α6 subunit immunoreactivity (79 ± 7%) and BZ-IS sites retained by the anti-α6(1–16 Cys) Fab antibody affinity column were the same.

In the pH 11.5 eluted fractions, again, BZ-S and BZ-IS [3H]Ro 15-4513 binding activity were both assayed. The results were difficult to quantify because of the low level of total [3H]Ro 15-4513 binding activity, but it was observed that flunitrazepam displaced a significant proportion of the total binding activity (see below). There was no significant retention of specific [3H]flunitrazepam binding sites by the anti-α6(1–16 Cys) antibody column. In agreement, [3H]flunitrazepam binding to the purified receptors was not detectable (n = 2, results not shown).

To determine the other GABA_A receptor polypeptides that copurified with the α6 subunit-containing GABA_A receptors, the pH 11.5 fractions 2–4 were pooled and analyzed by immunoblottting. Fig. 2 shows the results, where it can be seen that α1, β2/3, γ2, and δ subunit immunoreactivities were found. As for the bovine preparations, there was no detectable α2 or α3 subunit immunoreactivities. Note that we were unable to use the anti-β3(379–393) antibody (Pollard et al., 1991); this was raised to the bovine β3(379–393) sequence. The homologous rat sequence has three amino acid differences, and the rat β3 subunit is not recognized by the bovine antibody. The β subunits were thus identified by the monoclonal antibody bd-17, which recognizes both rat β2 and β3 subunits.

Quantification of the Percentage Coassocaiton of the α1 and α6 GABA_A Receptor Subunits in Adult Rat Cerebellum—The coassocation of the α1 and α6 subunits was investigated further and quantitatively by using different immunoaffinity columns in series. Thus GABA_A receptors were first purified by anti-α6(1–16 Cys) Fab antibody affinity chromatography. The receptor-containing fractions were pooled and applied to a sheep anti-α1(413–429) whole antibody affinity column. The affinity column filtrate and the pH 11.5 eluted fractions were both assayed. The results are shown in Fig. 4. The results are the means for three different double immunopurifications with duplicate immunoblots for each experiment.
summarizes the results obtained from three separate purifications. It demonstrates the coelution of the α1 and α6 subunit immunoreactivities. The results for the quantification of the immunoblots are summarized in Table III. For these experiments, equal volumes were applied for each sample thus permitting direct comparisons following densitometry. It was found that 79 ± 7% of the applied α6 subunit immunoreactivity was retained initially by the anti-α6 subunit antibody column. On application to the second different specificity antibody column, 41 ± 12% α6 subunit immunoreactivity was retarded (n = 2).

To determine the ratios of the α1–α6 subunits in the double purified receptor preparations, two sets of experiments were carried out. First, the primary α1 and α6 affinity-purified antibody dose dependences were determined, for a fixed antigen concentration, in immunoblots of double immunoaffinity-purified receptors (Fig. 4A). Second, using the antibody concentrations at saturation (Fig. 4A), the α6:α1 subunit-containing antigen was varied and the resultant immunoreactive bands quantified (Fig. 4B). The α1:α6 subunit ratio was 0.95 ± 0.1, which was the mean value for each antigen concentration and for n = 2 preparations. In immunoprecipitation assays (see below), it was found that the anti-α6(1–16 Cys) antibody did not pellet all the α6 subunit immunoreactivity even when used at saturation. Although immunoblotting and immunoprecipitation are two different experimental paradigms, further investigation was required to ensure that the α6 subunit was not being underestimated by using an antibody with low avidity. Thus, two immunoblots were carried out in parallel. In the first a single incubation with a saturating concentration of primary anti-α6(1–16 Cys) antibody was used. For the second immunoblot, this was processed as for the first except that the initial primary antibody was aspirated and the immunoblot then incubated with a fresh primary anti-α6(1–16 Cys) antibody at the same saturating concentration (Fig. 4A). Both immunoblots were then quantified by densitometry but no differences in the amount of α6 subunit immunoreactivity were found (results not shown).

Immunoblots of α6:α1 double immunoaffinity-purified receptors showed the coassociation of β2/3 and δ subunits (n = 1; results not shown), but attempts to show the localization of the γ2 subunit have so far been negative.

The Benzodiazepine Pharmacology of α1–α6 Subunit-containing GABA<sub>A</sub> Receptors—Since binding activity was not detectable in the α6:α1 double immunoaffiliated GABA<sub>A</sub> receptors, the benzodiazepine pharmacology of the α1–α6 subunit-containing receptors was investigated by quantitative immunoprecipitation using affinity-purified antibody at saturating concentrations. The results are summarized in Fig. 5. First, the α6 subunit antibody immunoprecipitated 23 ± 5% (n = 9) of the
total [\textsuperscript{3}H]Ro 15-4513 binding sites from solubilized rat cerebellar membranes. This value is in agreement with the percentage of total [\textsuperscript{3}H]Ro 15-4513 binding sites retained by the anti-\(\alpha_{6}(1-16\text{ Cys})\) antibody affinity column (Table II). It corresponded to 54 \pm 7\% of the BZ-IS sites but no significant immunoprecipitation of the BZ-S sites was found (values were 5 \pm 4\% see also Table II), again in agreement with results obtained from anti-\(\alpha_{6}(1-16\text{ Cys})\) Fab affinity chromatography (Table II). In contrast, the GABA\(_{A}\) receptor \(\alpha_{1}\) subunit immunoprecipitated 72 \pm 6\% of total [\textsuperscript{3}H]Ro 15-4513 sites. Significant immunoprecipitation of both BZ-S (77 \pm 6\%) and BZ-IS (64 \pm 10\%) sites was found (Fig. 5). These values agree with results obtained using anti-\(\alpha_{1}(413-429)\) immunoaffinity chromatography from adult rat cerebellum where 81 \pm 5\% of total, 85 \pm 10\% BZ-S, and 71 \pm 10\% BZ-IS [\textsuperscript{3}H]Ro 15-4513 binding sites were retained (\(n = 3\); results not shown).

The same immunoprecipitation assays were carried out on GABA\(_{A}\) receptors purified from adult rat cerebellum by anti-\(\alpha_{1}(324-341)\) antibody affinity chromatography. Here, the \(\alpha_{1}\) antibody immunoprecipitated close to 100\% of total, BZ-S, and BZ-IS [\textsuperscript{3}H]Ro 15-4513 sites as should be the case for an \(\alpha_{1}\) subunit-purified preparation. However, the \(\alpha_{6}\) subunit antibody immunoprecipitated a maximum of 30 \pm 5\% of total [\textsuperscript{3}H]Ro 15-4513 sites (i.e. \(\alpha_{1}\alpha_{6}\) subunit containing). When these binding sites were subfractionated into the BZ-IS and BZ-S sites, the values were not significant for the BZ-S but 47 \pm 7\% compared to a predicted 100\% for the BZ-IS sites. Increasing the antibody concentration did not effect the percentage of [\textsuperscript{3}H]Ro 15-4513 binding sites precipitated, but it was found that at these high antibody concentrations, \(\alpha_{6}\) subunit immunoreactivity was still present in the supernatant. Thus, the inability to immunoprecipitate all the BZ-IS [\textsuperscript{3}H]Ro 15-4513 sites may be attributed to the low avidity for the antibody. This has been encountered before for immunoprecipitations with the anti-\(\gamma_{2}(1-15\text{ Cys})\) antibody where the problem was overcome by sequential immunoprecipitations with fresh batches of antibody (Dugnan et al., 1992). This was not possible here because of the low levels of binding activity. Significantly, when the pharmacology of the immunoprecipitated pellet was determined directly instead of as a percentage of the total starting activity, 91 \pm 10\% (\(n = 2\)) of the [\textsuperscript{3}H]Ro 15-4513 binding was BZ-IS.

**DISCUSSION**

In this paper, we have described the purification of \(\alpha_{6}\) subunit-containing GABA\(_{A}\) receptors with the retention of their [\textsuperscript{3}H]Ro 15-4513 radioligand binding activities. We have sub-

![Image](5x.png)
stantiated the coexistence of the α1 and α6 subunit in single receptor oligomers and, in addition, we have quantified their percentage coassociation. In double immunofluorescence-purified receptors, the α1:α6 subunit ratio was 1:1 and the benzodiazepine pharmacology of this subset of receptors was BZ1S. Thus the use of the α6 and α1 immunoaffinity columns in series not only proved the coexistence of these two gene products in one receptor (41% of the α6 subunit receptor population, Table III) but also showed that in the rat cerebellum, either single variant α1 or α6 subunit-containing receptors exist. These results are in agreement with the emerging pattern from several groups. That is, that at least for the GABA$_A$ receptor α subunits, different isoforms do partially coexist within the same receptor molecule (summarized by Stephenson, 1995). With specific reference to the α1 and α6 subunits, the findings herein are in agreement with the localization of α1 and α6 GABA$_A$ receptor subunit-like immunoreactivities at the electron microscopic level where synapses in the cerebellum were found containing either α1 or α6 or both α1 and α6 polypeptides (Nusser et al., 1995). Moreover, Mathews et al. (1994) coexpressed α1, α6, β2, and γ2 polypeptides in mammalian cells and showed that the resultant pharmacological properties were distinct from both α1β1γ2 and α6β1γ2 receptors and best explained by an α1β6δ2γ2 hybrid receptor. Quirk et al. (1994), however, found no evidence for coassociation of α1 and α6 subunits but this may be explained by low avidity antibodies. Similarly, Korpi and Luddens (1993) found no evidence for the coassociation of all four subunits following transient expression in mammalian cells.

For the non-α subunits coassociated with the α6 polypeptide, the results reported here are in agreement with previous reports, where the coassociation of α6 with γ2 (Khan et al., 1994; Quirk et al., 1994), δ (Quirk et al., 1994), and β2/3 (Khan et al., 1994) was found. But Quirk et al. (1994) identified α6β2 and α6δ as two distinct populations, where the latter did not bind $[^3H]Ro$ 15-4513. In the α1α6 double immunofluorescence-purified receptors, we were unable to detect the GABA$_A$ receptor γ2 subunit by immunoblotting. Negative results here are difficult to interpret definitively because they may be explained by both the low levels of purified receptor and antibody avidity, a particular problem with the anti-γ2 subunit antibody used (cf. Stephenson et al., 1990; Duggan et al., 1992). But, it may also be that the γ2 subunit is associated with single α6 variant receptors. Consequently, the (α1α6δβ2γ3) receptor identified here may be similar to α6δ6 receptors described by Quirk et al. (1994), which do not bind $[^3H]Ro$ 15-4513. Further analysis of the anti-α1(413–429) post-column filtrate should clarify this.

The direct determination of the number of α subunits (a 1:1 ratio for α1:α6, therefore predicting two per receptor) is the first for native GABA$_A$ receptors. It is in agreement with the 1:1 ratio predictions for native receptors where the coexistence of two but not three different α subunits were detectable (Duggan et al., 1991), the inferred subunit complement of native cerebellar receptors, α1α6δβ2γ2, (Khan et al., 1994), and the subunit complement of an (α1)(α6)(δ1)(γ2) cloned receptor (Backus et al., 1993). The quantification described in this paper is not ideal because antibody molecules are bivalent and the antibodies used are polyclonal albeit to a restricted epitope. Thus it is a possibility that the number of antibodies bound per subunit may not be stoichiometric. However, this is unlikely because 1) steric hindrance would reduce the probability of two antibody molecules binding to different epitopes within the restricted 16-amino acid peptide sequence, and 2) the binding of one antibody molecule to two subunits would have an equal probability for the α1 and α6 subunits following reduction and denaturation in SDS-PAGE.

The study of cloned, single α GABA$_A$ receptors showed that the benzodiazepine subpharmacology was dependent on the type of α subunit (Luddens and Wisden, 1991). The α6 subunit-containing cloned receptors show BZ1S pharmacology in contrast to α1 receptors, which are BZ5 (Luddens et al., 1990). A single point mutation in the α6 subunit, Δ6R100H, results in a mutant receptor with high affinity for the classical benzodiazepines (Wieland et al., 1992). From the results reported herein, for (α1α6) receptors, the α6 subunit dominates the pharmacology yielding BZ1S pharmacology. This would be in agreement with Mathews et al. (1994).

Conclusions—In conclusion, we have demonstrated a direct correlation between the BZ1S, $[^3H]Ro$ 15-4513 binding site pharmacology and native, α6 subunit-containing GABA$_A$ receptors. Furthermore, quantitative results showed that 41% of all the α6 subunit-containing receptors coexist with an α1 subunit and that in these receptors, the α6:α1 ratio was 1:1 and they displayed BZ1S pharmacology. The functional significance of the extensive GABA$_A$ receptor heterogeneity remains to be solved. However, the fact that we find all combinations of association of these two polypeptides in percentages that preclude the random association of actively transcribed genes suggests that there may be an active sorting/assembly mechanism to form functional receptor subtypes.

REFERENCES
Backus, K. H., Arigoni, M., Drescher, U., Scheurer, L., Malherbe, P., Mohler, H., and Benson, J. A. (1993) NeuroReport 5, 285–288
Benke, D., Mertens, S., Treczek, A., Gilissen, L., and Mohler, H. (1991) J. Biol. Chem. 266, 4478–4483
Duggan, M. J., Pollard, S., and Stephenson, F. A. (1991) J. Biol. Chem. 266, 24778–24784
Duggan, M. J., Pollard, S., and Stephenson, F. A. (1993) Neuron 58, 72–77
Ewert, M., Shivers, B. D., Luddens, H., Mohler, H., and Seeburg, P. H. (1990) J. Cell Biol. 110, 2043–2048
Kern, W., and Sieghart, W. (1994) J. Neurochem. 62, 764–769
Khan, Z. U., Gutierrez, A., and De Blas, A. L. (1994) J. Neurochem. 63, 371–374
Korpi, E. R., and Luddens, H. (1993) Mol. Pharmacol. 44, 87–92
Luddens, H., and Wisden, W. (1991) Trends Pharmacol. Sci. 12, 49–51
Luddens, H., Pritchett, D. B., Kohler, M., Kilillisch, I., Keenan, K., Manyer, H., Sprengel, R., and Seeburg, P. H. (1990) Nature 346, 648–651
MacDonald, R. L., and Olsen, R. W. (1994) Annu. Rev. Neurosci. 17, 569–602
Mathews, G. C., Bledsoe-Sy, A. M., Holland, K. D., Isenberg, K. E., Covey, D. F., Tronci, J. A., and Ritschman, S. M. (1994) Neuron 13, 149–158
McKernan, R. M., Quirk, K., Prince, R., Cox, P. A., Gillard, N. P., Ragan, I. C., and Whiting, P. J. (1991) Neuron 7, 667–676
Mertens, S., Benke, D., and Mohler, H. (1993) J. Biol. Chem. 268, 5965–5973
Naveen, N., Green, T. P., Smith, I. L., and Barnard, E. A. (1994) J. Neurochem. 62, 815–818
Nusser, Z., Pollard, S., Thompson, C. L., Stephenson, F. A., Sieghart, W., and Somogyi, P. (1993) Brain Res. Assoc. Abstr. 12, 114
Pollard, S., Duggan, M. J., and Stephenson, F. A. (1991) FEBS Lett. 295, 81–83
Pollard, S., Duggan, M. J., and Stephenson, F. A. (1993) J. Biol. Chem. 268, 3753–3757
Quirk, K., Gillard, N. P., Ragan, I. C., Whiting, P. J., and McKernan, R. M. (1994) J. Biol. Chem. 269, 16020–16028
Sieghart, W., Eichenger, A., Richards, J. G., and Mohler, H. (1987) J. Neurochem. 48, 46–52
Stephenson, F. A. (1995) Biochim. J. 310, 1–9
Stephenson, F. A., and Duggan, M. J. (1991) in Molecular Neurobiology: A Practical Approach (Wheal, H., and Chad, J., eds) pp. 183–204, IRL Press, Oxford
Stephenson, F. A., Duggan, M. J., and Pollard, S. (1990) J. Biol. Chem. 265, 21160–21165
Thomas, C. L., Bodewitz, G., Stephenson, F. A., and Turner, J. D. (1992) Neurosci. Lett. 144, 53–56
Uusi-Oukari, M. (1992) J. Neurochem. 59, 568–573
Wieland, H. A., Luddens, H., and Seeburg, P. H. (1992) J. Biol. Chem. 267, 1426–1429