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The $GABA_A$/benzodiazepine receptor is a heterotetramer of homologous α and β subunits

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The $GABA_A$ receptor has been purified to homogeneity from bovine cerebral cortex. Under stringent conditions of isolation, the $GABA_A$ receptor was shown to consist only of α (M_r 53 000) and β (M_r 57 000) subunits. A densitometric scan of SDS–PAGE gels under reducing conditions showed that these subunits were present in a 1:1 ratio. A model of the receptor as a heterologous tetramer $\alpha_2\beta_2$ is proposed. Monoclonal antibodies have been raised to the purified bovine $GABA_A$ receptor. One of these antibodies, 1A6, was shown to react with both the α and β subunits of the purified receptor. The subunits were still positive in immunoblots following the removal of the carbohydrate moieties of the respective polypeptides by endoglycosidase F treatment. This antibody has been employed to demonstrate antigenic cross-reactivity between the $GABA_A$ receptors of three vertebrate species. It is further proposed that there is partial amino acid sequence homology between the α and β polypeptides and hence that they are derived from a single ancestral gene.

Key words: antibodies/benzodiazepines/GABA/receptor

Introduction

The brain $GABA_A$ receptor is a ligand-gated anion channel, the properties of which can be modulated by two important classes of drugs, the anxiolytic ('tranquillizer') benzodiazepines and the depressant barbiturates (Olsen, 1982). Such a receptor protein from mammalian brain, possessing specific binding sites for GABA and for these modulators and the predicted allosteric interactions between those sites, has been completely purified (Sigel *et al.*, 1983; Sigel and Barnard, 1984) and shown to be an acidic membrane glycoprotein containing α subunits (M_r 53 000) and β subunits (M_r 58 000), although there has not been general agreement on the precise subunit composition. Photoaffinity labelling with specific ligands applied to this pure $GABA_A$/benzodiazepine receptor has shown that the benzodiazepine binding site is located on the α subunit (Sigel *et al.*, 1983; Kirkness and Turner, 1986; Stephenson *et al.*, 1986b), whereas the binding site for GABA ligands resides on the β subunit (Casalotti *et al.*, 1986). Recently, Schoch *et al.* (1985) and Haring *et al.* (1985), describing monoclonal antibodies directed against the $GABA_A$/ benzodiazepine receptor, similarly found only α and β subunits in the receptor from bovine and human brain; these antibodies distinguished four epitopes of the receptor protein which were either α subunit-specific or β subunit-specific or were not seen at all in immunoblots, the latter type being conformation-dependent. We now report the production of another class of monoclonal antibody which recognizes both the α and β polypeptides in the mammalian GABA/benzodiazepine receptor (and not their carbohydrate attachments, whose sizes we estimate here). We propose that the α and β subunits show limited but definite structural homology and that these two polypeptides are derived from a single ancestral gene. The $GABA_A$/benzodiazepine receptor is shown to have the structure $\alpha_2\beta_2$.

Results

The $GABA_A$ receptor used in this study was purified from bovine cerebral cortex by the Na^+ deoxycholate/Triton X-100 method (Sigel *et al.*, 1983). In this preparation, the purified receptor was shown to have a high affinity specific binding site for GABA agonists and antagonists, a high affinity specific binding site for the benzodiazepine agonists, antagonists and inverse agonists, and also a low affinity GABA binding site detected by the GABA agonist facilitation of benzodiazepine binding. The receptor preparation was homogeneous in several separation systems, one of which was narrow-range isoelectric focusing, where a single band of pI 5.6 was found (Stephenson *et al.*, 1986a). SDS–polyacrylamide gels of the isolated $GABA_A$ receptor carried out under reducing conditions and stained with either Coomassie Blue or silver showed the presence of only the α and β subunits (Figure 1a,b). A quantitative scan showed that these were present in a 1:1 stoichiometry (Figure 1b). At low [^{3}H]flunitrazepam concentration, i.e. ~5 nM, the α subunit exclusively is specifically photoaffinity labelled (Figure 1a, track D).

In the production of monoclonal antibodies directed against the $GABA_A$ receptor, typically from a single fusion, 10 wells containing antibody-secreting hybrid cells were positive in the ELISA assay using the solid-phase receptor screen. The cells from four of these were cloned successfully with the production of one positive clone. This antibody, 1A6, was shown to be of the IgM class by ELISA assay using rabbit peroxidase-conjugated anti-mouse class-specific antibodies (Serotec). The 1A6 bound specifically and to saturation to the immobilized purified bovine $GABA_A$ receptor (Figure 2), with binding detectable at a dilution of 1:3000 of the culture supernatant and half-maximal binding exhibited at a dilution of 1:500 of the supernatant from a confluent culture of the 1A6-producing cells.

The mouse serum which was collected immediately before the hybridoma fusion which yielded 1A6, recognized both the α and β subunits in immunoblots and that monoclonal antibody itself was also strongly reactive with both the subunits (Figure 1c, track A). However, the α subunit reacted more strongly with antibody 1A6 than did the β subunit, because in immunoblots where a series of decreasing concentrations of the receptor were applied the α subunit staining persisted at lower antigen concentrations than the β subunit (e.g. Figure 1c, track B). In preparations where the receptor was purified under slightly less stringent conditions, an additional polypeptide of M_r 46 000 was present by protein staining; this was also positive in the immunoblots (Figure 1c, track G). When preparations of the parent membranes were employed as the antigen in the immunoblots, only the α subunit

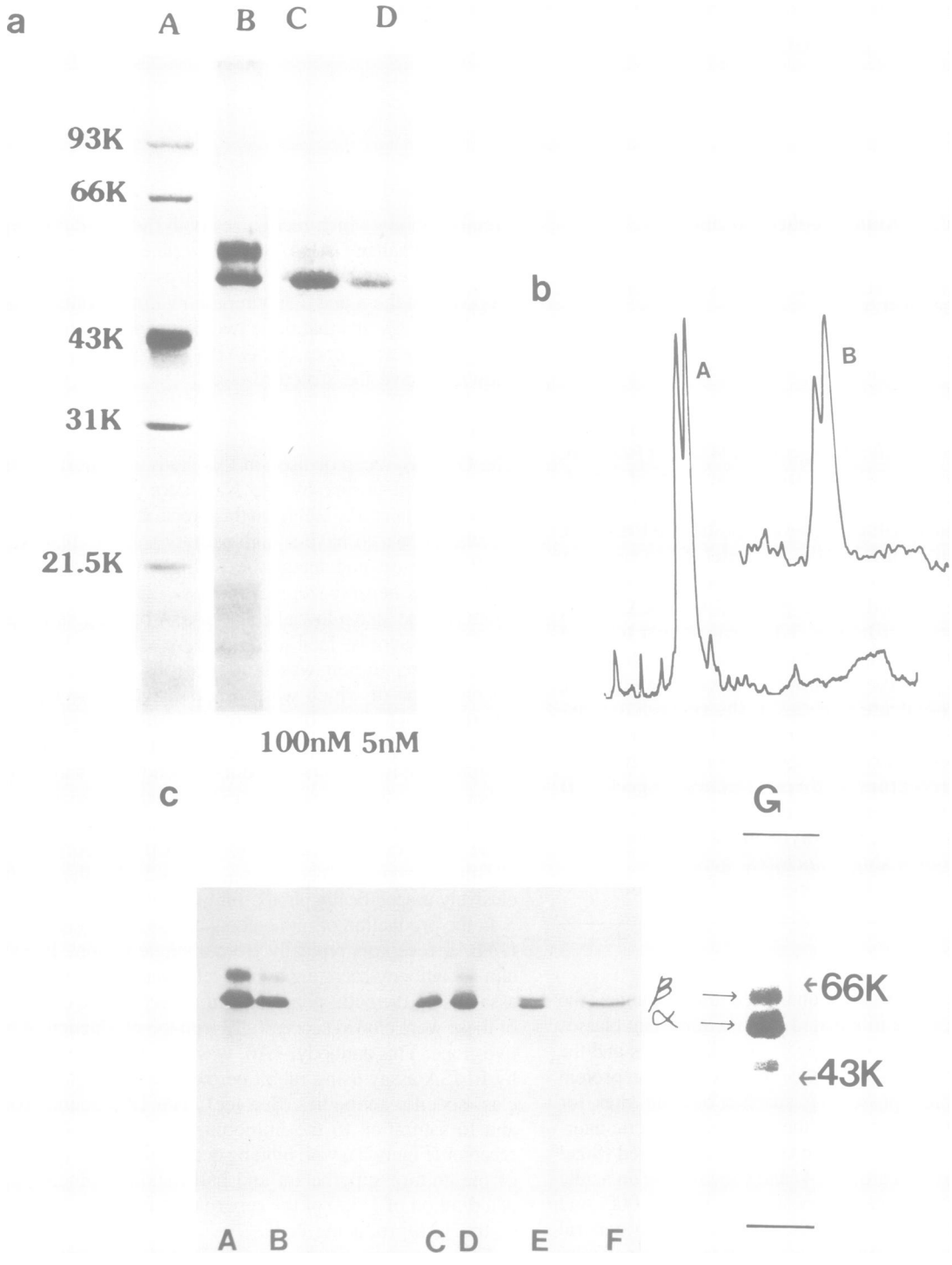

Fig. 1. SDS−PAGE of purified $GABA_A$ receptor preparations. The receptor was purified and SDS−PAGE under reducing conditions was carried out exactly as described in Materials and methods. **(a) Lane A:** standards (as used in calibrating all of the separations in this figure) of phosphorylase b, bovine serum albumin, ovalbumin, carbonic anhydrase and soybean trypsin inhibitor, with their M_r values marked (silver stained). **Lane B:** purified receptor (silver stained). **Lane C:** purified receptor which has been photoaffinity-labelled for 6 min at 0°C by 100 nM [^{3}H]flunitrazepam (fluorograph, exposed to the X-ray film at −80°C for 15 days). **Lane D:** as **lane C**, but using 5 nM [^{3}H]flunitrazepam. Controls (not shown) using the same conditions but in the dark, or with 70 μm unlabelled flunitrazepam also present, gave completely blank fluorographs. **(b)** Densitometer (Joyce−Loebel) scans of photographic negatives (in linear response conditions) of gels containing the receptor purified in CHAPS medium. **(A)** Stained (below saturation level) with silver. (The noise as seen in a scan of the adjacent gel background has not been subtracted.) **(B)** Stained with Coomassie Blue. Computer integration of the overlapping peaks seen of the α and β subunits yielded in replicate scans a mean ratio of 0.93:1 (α:β) for the silver stain and 1.12:1 (α:β) for the dye. **(c)** Immunoblots probed with antibody 1A6. The samples were prepared as described in Materials and methods and are as follows: **lane A**, $GABA_A$ receptor (8.7 pmol) purified from bovine cerebral cortex by Na^+ deoxycholate extraction of brain membranes followed by the use of 0.6% (w/v) CHAPS as the only detergent in all subsequent chromatographic procedures. **Lane B**, $GABA_A$ receptor (4.3 pmol) purified exactly as for **lane A**. **Lane C**, $GABA_A$ receptor (4 pmol) purified from adult rat brain minus cerebellum by the Na^+ deoxycholate/Triton X-100 method. **Lane D**, as for **lane C** but the amount of rat $GABA_A$ receptor loaded was 11 pmol. **Lane E**, $GABA_A$ receptor (4 pmol) purified from 1-day-old chick brain by the procedure as in **lanes A** and **B** above. **Lane F**, membranes prepared from bovine cerebral cortex. **Lane G**, $GABA_A$ receptor purified from bovine cerebral cortex as for **lane A** except that soybean trypsin inhibitor and ovomucoid inhibitor were not present in the extraction buffers. **Lane G** is taken from a separate immunoblot, hence two markers are shown.

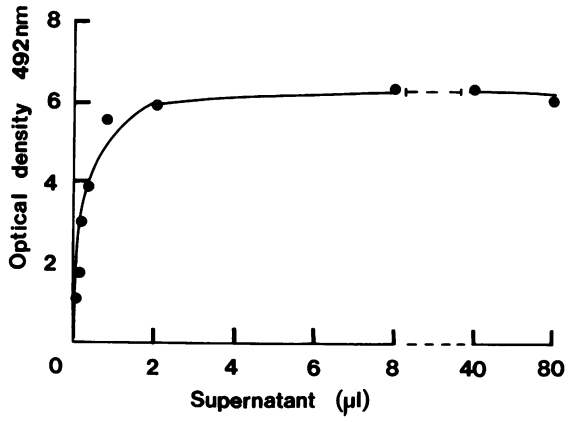

Fig. 2. The binding of antibody 1A6 to the purified immobilized $GABA_A$ receptor. An ELISA assay was carried out exactly as described in Materials and methods with the purified $GABA_A$ receptor immobilized on the ELISA wells as the antigen and with increasing volumes of supernatant taken from a confluent culture of the 1A6-producing hybridoma cells. The half-maximal absorbance obtained was from a 1:500 dilution of the respective supernatant.

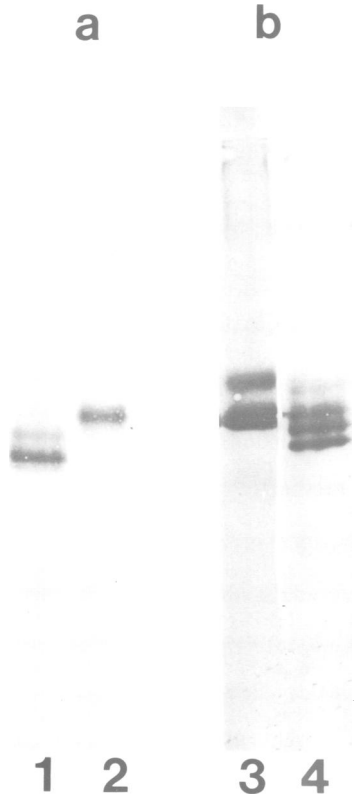

Fig. 3. Immunoblots before and after deglycosylation of the purified receptor. **(a)** Purified $GABA_A$ receptor (~5 pmol [^{3}H]muscimol binding sites) was precipitated with trichloroacetic acid and washed with acetone as described in Materials and methods. The protein sample was where stated deglycosylated as described in Materials and methods with endoglycosidase F. Immunoblotting was performed with the rabbit anti-(bovine $GABA_A$ receptor) α subunit-specific polyclonal antibody as previously described (Stephenson *et al.*, 1986a). **Lane 1** is deglycosylated $GABA_A$ receptor; **lane 2** is a control sample treated identically to that in **lane 1**, i.e. incubation overnight at 37°C, but in the absence of enzyme. **(b)** Samples were prepared exactly as for **(a)** except that the immunoblotting was carried out using the monoclonal antibody 1A6. **Lane 3**, control receptor incubated overnight at 37°C in the absence of endoglycosidase F; **lane 4**, deglycosidated receptor. For both **(a)** and **(b)**, an identical pattern to the Western blot was obtained by silver staining of the endoglycosidase-treated receptor, except that a band of M_r ~22 000 was present which was identified as the enzyme.

was clearly revealed by the antibody reaction under comparable conditions (Figure 1c, track F).

Since both the α and β subunits contain covalently linked oligosaccharide, it is conceivable that the monoclonal antibody 1A6 specifically recognizes a carbohydrate moiety that is common to both the subunits. Thus, deglycosylation of the purified receptor was carried out using endoglycosidase F treatment and the products were detected by immunoblotting. After this treatment, it was found with an α subunit-specific polyclonal antibody (Stephenson *et al.*, 1986a) that the α subunit disappeared and a major product about 8000 lower in M_r than the original α (M_r in the range 44−46 000, in three experiments) was detected in parallel with a minor product about 5000 lower in M_r than α (M_r in the range 48−49 000) (Figure 3a). When the final products from this treatment of the intact, denatured receptor were revealed by antibody 1A6, these, plus two additional products of M_r 57 000 (minor) and 55 000 derived from the β subunit were found (Figure 3B). The same pattern was obtained with silver-stained polyacrylamide gels of the purified enzymically deglycosylated bovine $GABA_A$ receptor. Similar results were also obtained (not shown) following treatment of the purified receptor with trifluoromethane sulphonic acid, which cleaves off carbohydrate peripheral groups, both *O*-linked and *N*-linked (Edge *et al.*, 1981). The final products were then of M_r 55 000 for the β subunit and M_r 44 000 for the α subunit.

The antibody 1A6 was also used to demonstrate homology between the $GABA_A$ receptors from several vertebrate species. When the receptor was purified in the CHAPS medium from rat cerebral cortex and from 1-day-old chick brain, each source yielded a protein with subunits of essentially the same sizes as for the bovine receptor except that the chick brain receptor α subunit showed a distinct doublet (Stephenson, 1987; F.A. Stephenson, unpublished). For the purified rat receptor, both the α and β subunit are recognized by 1A6, but its binding to the β subunit is lost at higher receptor concentrations than in the bovine case (Figure 1c, tracks C and D). In the case of the chick receptor, both the components of the α subunit are positive in immunoblots with 1A6; the β subunit, however, is not recognized by the antibody (Figure 1c, track E). This was seen even at chick receptor concentrations where the β subunit reaction is strong in the purified bovine $GABA_A$ receptor protein.

Discussion

In this paper we have presented evidence that the mammalian $GABA_A$ receptor has a heterologous structure with stoichiometry $\alpha_2\beta_2$. Further, we have described a monoclonal antibody, 1A6, which recognizes both the α and β subunits in immunoblots. Hence, we propose that these subunits have amino acid sequence homology and that the antigenic determinant involved is common also to the $GABA_A$ receptor of other vertebrate species.

The deduction of the $\alpha_2\beta_2$ structure is based upon the equal content of the subunits (Figure 1a and b) and the determination of the mol. wt of the oligomeric native protein. This was calculated from hydrodynamic measurements of the purified protein: it was found that, after the correction for bound detergent by the H_2O/D_2O method, the mol. wt of the isolated protein was ~230 000 (Stephenson *et al.*, 1986b; C.Mamalaki *et al.*, unpublished results). The mol. wt of the deglycosylated α and β subunits are 44 000 and 55 000 respectively as calculated from SDS−PAGE. However, it should be noted that these are apparent values only and are subject to some residual error inherent in the estimation by SDS gel electrophoresis of the M_r of membrane proteins. Thus the structure $\alpha_2\beta_2$ for the native $GABA_A$

receptor is within the limit of experimental error for the mol. wt value of the complex. For comparison, we can note that under some conditions of radiation inactivation, essentially the same size (220 000) is found by that technique for this receptor in mammalian brain membranes (Chang and Barnard, 1982).

In the deglycosylation experiments it was found that following treatment of the purified receptor at lower endoglycosidase F concentration, an intermediate polypeptide was found with M_r 49 000. The reason for the formation of this intermediate is unclear: it could be due to a glycosylation site (e.g. in the second oligosaccharide chain) having some resistance or inaccessibility to the endoglycosidase, giving rise to a stable partly deglycosylated intermediate, or to another subunit hitherto not detected because it completely co-migrates with α in the bovine receptor in the native, i.e. non-deglycosylated, state. The former type of explanation and not the latter is more favoured for the following reasons: (i) the labelling of the α subunit seen in the gels is always co-extensive for protein staining (silver or Coomassie Blue) and the [^{3}H]flunitrazepam photo-reaction (Figure 1a); (ii) the bovine, rat and human receptors each show in immunoblots a single sharp α subunit band of the same size (Figure 1c and Schoch *et al.*, 1985); (iii) the chick $GABA_A$ receptor α subunit shows two components but these are both positive with 1A6 (Figure 1c) as well as (data not shown) with the bovine α-specific polypeptide antibody (Stephenson *et al.*, 1986a) or with the [^{3}H]flunitrazepam photoaffinity labelling, while the chick β subunit negative in all of these reactions is still seen at M_r 58 000 in protein staining.

The deglycosylation experiments prove that it is the α and β polypeptides themselves and not their carbohydrate attachments that contain the common epitope recognized by 1A6. The epitope, however, is not identical between the two subunits because when the α and β subunits are present in gels with equal stoichiometry, the α subunit always reacts more strongly with antibody 1A6 than the β subunit. This finding is compatible with, for example, a single amino acid replacement between the two epitopes and it is thought that this is why, in the parent membranes, only the α subunit was clearly revealed by the antibody reaction under comparable conditions (Figure 1c, track G). For the weaker-reacting β subunit, the concentration obtainable in the gel from application of an SDS-solubilized membrane sample is presumed to be too low to be readily detectable by immunoblotting.

It is of interest to note here that the only ligand-gated receptor channel whose structure has been definitively determined so far, namely the nicotinic acetylcholine receptor of electric organ or skeletal muscle, has four types of subunit. These subunits are homologous to the extent of 32–50% for different pairs within one species and descent from a single ancestral subunit fits well with the observed differences (Kubo *et al.*, 1985). A large number of monoclonal antibodies has been raised against the purified nicotinic acetylcholine receptor (native or denatured) but only a small fraction (<5%) recognize strongly more than one of its subunit types (Sargent *et al.*, 1984; Lindstrom, 1984). So far, of 23 monoclonal antibodies to the $GABA_A$ receptor known to us or reported upon, only the 1A6 described herein shows inter-subunit cross-reactivity. We deduce, on the basis of all the features noted here, that the α and β subunits of this receptor are likewise related to each other, structurally and phylogenetically.

The deduction of the $\alpha_2\beta_2$ model for the $GABA_A$ receptor is based on structural observations. Since the α subunit has been identified as the benzodiazepine binding polypeptide and, more recently, the β subunit as the polypeptide that carries the binding site for GABA (Casalotti *et al.*, 1986), it is now possible to correlate the distribution of the respective binding sites with the $\alpha_2\beta_2$ structure. Only in one case, however, has the number of reversible high-affinity GABA binding sites been shown to equal the number of reversible benzodiazepine binding sites. That observation was made when the $GABA_A$ receptor was extracted from brain membranes with CHAPS and immunoprecipitation was carried out of both the aforementioned reversible binding sites (in the absence of endogenous GABA) with a polyclonal anti-($GABA_A$ receptor) antibody (Stephenson *et al.*, 1986a). This was a single point determination corrected to the B_{max} value. In the presence of CHAPS the dissociation constant for muscimol binding is difficult to determine accurately because CHAPS itself can inhibit this binding activity (Sigel and Barnard, 1984). In other detergent extraction and purification conditions the ratio has been reported to be in the range of two to four GABA sites to one benzodiazepine binding site (Sigel *et al.*, 1983; Fischer and Olsen, 1986; Kirkness and Turner, 1986). The presence of two β subunits and therefore of two high-affinity GABA binding sites per receptor oligomer is in accord with the GABA concentration dependence of the channel-opening activity in neuronal patch-clamp studies, where it has been found that an agonist occupancy number of two is required for function (Sakmann *et al.*, 1983; Bormann and Clapham, 1985; Smart *et al.*, 1987).

The possible discrepancy between the reported number of the reversible benzodiazepine binding sites and the $\alpha_2\beta_2$ structure postulated requires further investigation.

It should be noted also that the $\alpha_2\beta_2$ structure proposed has not yet been proven to contain the GABA-gated anion channel. The final proof that this structure $\alpha_2\beta_2$ is sufficient for all of the activities of the native $GABA_A$ receptor/channel will come from application of the *Xenopus* oocyte translation system, already shown to be capable of expressing that channel (so far, from impure mRNAs, Smart *et al.*, 1983), when one is able to inject the pure mRNAs encoding the α and β subunits.

Materials and methods

Reagents

[*N*-methyl-^{3}H]flunitrazepam (85 Ci/mmol), [methylene-^{3}H]muscimol (9 Ci/mmol), biotinylated anti-(rabbit IgG), biotinylated anti-(mouse Ig) and streptavidin-biotinylated peroxidase were all from Amersham International, UK. [^{35}S]t-butylbicyclophosphorothionate (TBPS) (70–90 Ci/mmol) and unlabelled TBPS were from New England Nuclear. Endoglycosidase F ([peptide-N^4[*N*-acetyl-β-glucosaminyl] asparagine amidase) from *Flavobacterium meningosepticum* was purchased from Genzyme Corp., Boston, MA. Flunitrazepam and Ro 7-1986/1 were gifts from Hoffman-La Roche, Basle, Switzerland and chlorazepate was a gift from Boehringer Ingelheim, Bracknell, UK. Other materials not specified were as given previously (Sigel *et al.*, 1983; Sigel and Barnard, 1984).

$GABA_A$ receptor purification and characterization

The $GABA_A$ receptor was purified from bovine cerebral cortex, by Ro 7-1986/1–agarose affinity chromatography as previously described (Sigel *et al.*, 1983) except that soybean trypsin inhibitor (10 μg/ml) and ovomucoid ovoinhibitor (10 μg/ml) were present until the application of the Na^+ deoxycholate extract to the affinity column. When the $GABA_A$ receptor was purified for animal immunization, the Triton X-100 concentration was reduced to 0.05% (v/v) in the ion-exchange chromatography step. Radioligand binding was measured by the bovine gamma globulin/polyethylene glycol precipitation/filtration assay (Sigel *et al.*, 1983). SDS–PAGE was carried out using 10% polyacrylamide slab gels with silver or Coomassie Blue staining as before (Sigel *et al.*, 1983). Samples analysed by SDS–PAGE were firstly precipitated with 12% (w/v) trichloroacetic acid and acetone washed (Sigel *et al.*, 1983) or were precipitated by the $CHCl_3$/MeOH method of Wessel and Flugge (1984) prior to boiling for 5 min in the presence of 100 mM DTT.

Enzyme-linked immunosorbent assay (ELISA) for the detection of anti-($GABA_A$ receptor) antibodies. The ELISA assay was based on the method of Kendall *et al.* (1983). Wells of polethylene microtitre plates (Becton Dickinson, Oxnard,

CA) were incubated overnight at 4°C with purified bovine $GABA_A$ receptor (60 μl, 3–5 μg protein/ml) diluted in phosphate-buffered saline (PBS). The coated cells were washed three times with 200 μl of 0.25% (w/v) gelatin in PBS (PBS/gelatin) followed by an incubation for 45 min at 37°C with the same solution. The wells were incubated with hybridoma culture supernatant (80 μl) for 1 h at 37°C followed by 3 × 200-μl washes with PBS/gelatin and one PBS/gelatin wash (200 μl) for 10 mn at 37°C. Biotinylated anti-mouse Ig was diluted 1:750 with PBS/gelatin and the wells were incubated with this solution (80 μl) for 2 h at 37°C. The wells were washed four times with PBS/gelatin, incubated with streptavidin-biotinylated peroxidase (200 μl) diluted 1:1000 with PBS/gelatin for 45 min at 37°C and washed a further three times with PBS/gelatin and once with PBS only. The reaction was developed with a solution which contained 0.04% (w/v) *o*-phenylene diamine and 0.003% H_2O_2 (80 μl) in H_2O. It was terminated by the addition of 20% H_2SO_4 (80 μl) and the absorbance at λ = 492 nm was measured in an ELISA Titertek Multiscan photometer.

Monoclonal antibody production

The purified $GABA_A$ receptor was dialysed overnight at 4°C against 20 mM K phosphate, pH 7.4, 0.1 mM EDTA, 0.05% Triton X-100 (2 × 1000 vol). The dialysate was lyophilized and resuspended in deionized water. This preparation was used for animal immunization. BALB/c mice (6-week-old females) were immunized i.p. with purified $GABA_A$ receptor (50 μg) emulsified in an equal volume of Freund's complete adjuvant. Booster injections of 10–15 μg $GABA_A$ receptor in the absence of adjuvant were administered 5–10 weeks later, four days prior to removal of the spleens. Hybridomas (Köhler and Milstein, 1975) were produced by the fusion of ~10^8 spleen cells in complete DMEM with ~10^7 Sp2 OAg myeloma cells. The culture supernatants were screened for the production of anti-($GABA_A$ receptor) antibodies as described above. The cells from the culture supernatants which were positive in the screening procedure were cloned three times by the limiting dilution method in 96-well plates, followed by a serial dilution as described previously (Mehraban *et al.*, 1984). The cloned hybrid was propagated in complete DMEM/HT.

Western blotting

Western blotting was carried out as previously described (Stephenson *et al.*, 1986a) except that the transfer buffer did not contain SDS and biotinylated anti-mouse Ig was used as the second antibody for the monoclonal antibodies.

Endoglycosidase F treatment

The purified $GABA_A$ receptor (~5 pmol [^{3}H]muscimol binding sites) was precipitated with 12% trichloroacetic acid and washed with acetone as described previously (Sigel *et al.*, 1983). The protein pellet was dissolved firstly in 1% SDS/0.2 M Na phosphate, pH 8.6 then incubated in 0.2% SDS, 10 mM phenanthroline, 1.25% Nonidet P-40 in 0.2 M Na phosphate, pH 8.6. Endoglycosidase F was added to one half of the incubation mixture to a final concentration of 2 U/ml and samples were incubated overnight at 37°C. The incubation mixtures were precipitated with 12% trichloroacetic acid as above and subjected to SDS–PAGE under reducing conditions. The products were transferred to nitrocellulose filters and Western blotting was performed as above.

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References

Bormann,J. and Clapham,D.E. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 2168–2172.

Casalotti,S.O., Stephenson,F.A. and Barnard,E.A. (1986) *J. Biol. Chem.*, **261**, 15013–15016.

Chang,L.-R. and Barnard,E.A. (1982) *J. Neurochem.*, **39**, 1507–1518.

Edge,A.S.B., Fattynek,C.R., Hof,L., Reichert,L.-E. and Weber,P. (1981) *Anal. Biochem.*, **118**, 131–137.

Fischer,J.B. and Olsen,R.W. (1986) In Venter,C. and Olsen,R.W. (eds), *Benzodiazepine/GABA Receptors and Chloride Channels*. Alan R.Liss, New York, pp. 241–259.

Haring,P., Stahli,C., Schoch,P., Takacs,B., Staehelin,T. and Möhler,H. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 4837–4841.

Kendall,C., Ionescu-Matiu,I. and Dreesman,G.R. (1983) *J. Immunol. Methods*, **56**, 329–339.

Kirkness,E.F. and Turner,A.J. (1986) *Biochem. J.*, **233**, 256–270.

Köhler,G. and Milstein,C. (1975) *Nature*, **256**, 495–497.

Kubo,T., Noda,M., Takai,T., Tanabe,T., Kayano,T., Shimizu,S., Tanaka,K., Takahashi,H., Hirose,T., Inayama,S., Kikuno,R., Miyata,T. and Numa,S. (1985) *Eur. J. Biochem.*, **149**, 5–13.

Lindstrom,J. (1984) In Venter,J.C., Fraser,C.M. and Lindstrom,J. (eds), *Monoclonal and Anti-Idiotypic Antibodies: Probes for the Receptor Structure and Function*. Alan R.Liss, New York, pp. 21–57.

Mehraban,F., Kemshead,J.T. and Dolly,J.O. (1984) *Eur. J. Biochem.*, **138**, 53–61.

Olsen,R.W. (1982) *Annu. Rev. Pharmacol. Toxicol.*, **22**, 245–277.

Sakmann,B., Hamill,O.P. and Bormann,J. (1983) *J. Neural. Transm. Suppl.*, **18**, 83–93.

Sargent,P.B., Hedges,B.E., Traveler,L., Clemmons,L., Tzartos,S. and Lindstrom,J.M. (1984) *J. Cell Biol.*, **98**, 609–618.

Schoch,P., Richards,J.G., Häring,P., Takacs,B., Stahli,C., Staehelin,T., Haefeley,W. and Möhler,H. (1985) *Nature*, **314**, 168–171.

Sigel,E. and Barnard,E.A. (1984) *J. Biol. Chem.*, **259**, 7219–7223.

Sigel,E., Stephenson,F.A., Mamalaki,C. and Barnard,E.A. (1983) *J. Biol. Chem.*, **258**, 6965–6971.

Smart,T.G., Constanti,A., Bilbe,G., Brown,D.A. and Barnard,E.A. (1983) *Neurosci. Lett.*, **40**, 55–59.

Smart,T.G., Houamed,K.M., Van Renterghem,C. and Constanti,A. (1987) *Trans. Biochem. Soc.* in press.

Stephenson,F.A. (1987) *J. Rec. Res.*, in press.

Stephenson,F.A., Casalotti,S.O., Mamalaki,C., and Barnard,E.A. (1986a) *J. Neurochem.*, **46**, 854–861.

Stephenson,F.A., Mamalaki,C., Casalotti,S.O. and Barnard,E.A. (1986b) *Biochem. Soc. Symp.*, **52**, 33–40.

Wessel,D. and Flugge,U.I. (1984) *Anal. Biochem.*, **138**, 141–143.

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