The γ -aminobutyrate/benzodiazepine receptor from pig brain

Purification and characterization of the receptor complex from cerebral cortex and cerebellum

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The γ -aminobutyrate/benzodiazepine-receptor complex has been purified from a Triton X-100 extract of crude synaptic membranes from pig cerebral cortex and cerebellum by a combination of affinity and ion-exchange chromatography. [3 H]Flunitrazepam binding activity was purified 2200-fold from cortex with an overall yield of 2%. The dissociation constants for the binding of [3 H]muscimol and [3 H]flunitrazepam to the receptor complex were 14 ± 3 nM and 14 ± 2 nM respectively. The ratio of [3 H]muscimol to [3 H]flunitrazepam binding sites was in the range 2.2–2.8. There appeared to be no selective inactivation of either binding site during the purification procedure. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis revealed two major polypeptides of M_r 49000 and 55000 from both cortex and cerebellum. When the receptor from cortex was photoaffinity labelled with [3 H]flunitrazepam, radioactivity was incorporated predominantly into the M_r -49000 polypeptide, although some radioactivity was detectable in the M_r -55000 band. The cerebellar receptor was photoaffinity labelled on the 49000- M_r polypeptide but not on the polypeptide of M_r 55000. In addition, some radioactivity was detected in a minor polypeptide of M_r 43000. When purified in the presence of 3-[(3-cholamidopropyl)dimethylammonio]propanesulphonate the same major polypeptide components (M_r 49000 and 55000) were isolated, but the receptor now retained its ability to be modulated by secobarbital and by the anaesthetic propanidid.

INTRODUCTION

γ -Aminobutyrate (GABA) is the principal inhibitory neurotransmitter in the mammalian central nervous system. A major class of GABA receptors are associated with Cl^- ion channels which constitute part of a protein complex containing 'receptor' sites for GABA, benzodiazepines, picrotoxinin and barbiturates (Olsen, 1981; Turner & Whittle, 1983). Recently, there have been several reports describing the purification of solubilized GABA/benzodiazepine receptors by affinity chromatography using benzodiazepines as immobilized ligands (Martini *et al.*, 1983; Sigel *et al.*, 1983; Sigel & Barnard, 1984; Taguchi & Kuriyama, 1984). Although, in all reports, GABA- and benzodiazepine-receptor-binding activities were found to co-purify, different preparations have displayed differences in subunit composition and ratio of ligand binding sites. In addition, some of the characteristic interactions between the recognition sites of the membrane-bound receptor complex have been reported to be retained after extraction and purification in media containing Triton X-100 (Martini *et al.*, 1983) and Chaps (Sigel & Barnard, 1984), but not deoxycholate/Triton X-100 (Sigel *et al.*, 1983). It would appear, therefore, that the nature of the detergent used during isolation is of critical importance to the binding characteristics of the purified receptor complex. In this respect, the ability of barbiturates to modulate ligand binding to the receptor complex appears to be particularly sensitive to the nature of the detergent used for solubilization.

Stimulation by barbiturates of solubilized GABA and benzodiazepine receptor binding has been demonstrated

only after extraction of membranes with Chaps (Stephenson & Olsen, 1982). GABA receptor binding to a Chaps-solubilized preparation is also enhanced by the anaesthetic propanidid [the preceding paper (Kirkness & Turner, 1986)]. When the GABA/benzodiazepine receptor was purified in the presence of Chaps, pentobarbital caused a weak stimulation of benzodiazepine binding, although an effect on GABA receptor binding was not reported (Sigel & Barnard, 1984).

Although early studies indicated central-type benzodiazepine receptors existed in brain as an homogeneous population (Squires & Braestrup, 1977), more recent evidence obtained from ligand-displacement studies (Klepner *et al.*, 1979; Nielsen & Braestrup, 1980) and analysis by photoaffinity labelling (Sieghart & Karobath, 1980) has suggested the possibility of receptor subtypes. The evidence supporting receptor heterogeneity can be demonstrated in tissue from several brain regions, including cortex and hippocampus, but not from cerebellum. In the present paper we compare characteristics of the GABA/benzodiazepine receptor complex isolated from both pig cerebral cortex and cerebellum after solubilization and purification in the presence of Triton X-100. We have also examined the effects of barbiturates and propanidid on GABA receptor binding to a Chaps-purified receptor preparation.

EXPERIMENTAL

Materials

[*methylene- 3 H*]Muscimol (20.6 Ci/mmol) was from New England Nuclear, [*N-methyl- 3 H*]flunitrazepam (74 Ci/mmol) was from The Radiochemical Centre

Abbreviations used: Chaps, 3-[(3-cholamidopropyl)dimethylammonio]propanesulphonate; GABA, γ -aminobutyrate; PEG, poly(ethylene glycol); SDS, sodium dodecyl sulphate.

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(Amersham, Bucks., U.K.), adipic acid dihydrazide-agarose, Chaps, L- α -lecithin (phosphatidylcholine), muscimol and secobarbital were from Sigma (Poole, Dorset, U.K.). All other materials were purchased from BDH, Poole, Dorset, U.K. Propanidol and chlorazepate were gifts from, respectively, Bayer U.K. Ltd. and Boehringer-Ingelheim (U.K.); other benzodiazepine compounds were donated by Hoffman-La Roche (Basel, Switzerland).

Preparation of solubilized receptor

Pig brain was obtained fresh from a slaughterhouse and the cortex or cerebellum removed, frozen and stored at -70°C until use. Soluble receptor was prepared essentially as described previously (Sigel & Barnard, 1984) with the following alterations. Washed membranes were solubilized in 2% Triton X-100, 5 mM-Chaps or 25 mM-Chaps/0.2% L- α -lecithin instead of the detergent used in the previous procedure. The membrane suspension was stirred with detergent for 30 min at 4°C before centrifugation at $140\,000\text{ g}$ for 1 h. The solubilized receptor was prepared in media containing the proteinase inhibitors EDTA (1 mM), benzamidine (1 mM), phenylmethanesulphonyl fluoride (0.3 mM), bacitracin (100 mg/litre) and trypsin inhibitors type II-S and II-O (10 mg/litre; Sigma).

Isolation of the receptor complex

Affinity chromatography and ion-exchange chromatography were performed essentially as described previously (Sigel *et al.*, 1983), with the following changes. After application of the Triton X-100-solubilized receptor complex to the affinity column, the column was washed at 50 ml/h with 10 mM-potassium phosphate (pH 7.4)/250 mM-KCl/2 mM-magnesium acetate/0.1 mM-EGTA/10% (w/v) sucrose/0.2% (w/v) Triton X-100 (800 ml). All other steps were as previously described. Receptor preparations solubilized with 5 mM-Chaps or 25 mM-Chaps/0.2% L- α -lecithin were purified identically, except that in all media used during purification, Triton X-100 was replaced by 5 mM-Chaps or 5 mM-Chaps/0.05% L- α -lecithin respectively.

Binding assays

Ligand binding to the solubilized receptor complex was measured by a PEG precipitation/sedimentation assay (Stephenson *et al.*, 1982). The receptor complex was diluted into an assay medium that contained 20 mM-potassium phosphate, pH 7.5, 100 mM-KCl, 0.1 mM-EDTA and either 0.1% Triton X-100 or 5 mM-Chaps. Unless stated otherwise, diluted receptor in a total volume of $200\text{ }\mu\text{l}$ was incubated with 40 nM-[^3H]muscimol or 30 nM-[^3H]flunitrazepam at 0°C for 30 or 45 min respectively. Non-specific binding was determined in the presence of $50\text{ }\mu\text{M}$ -muscimol or $30\text{ }\mu\text{M}$ -flunitrazepam. After incubation, 3.3% (w/v) γ -globulin (15 μl), followed by 36% (w/v) PEG (85 μl), both in assay medium, were added and the assay tubes vortex-mixed and centrifuged at $11\,000\text{ g}$ for 10 min. The pellets were rinsed twice superficially with ice-cold buffer. Rinsed pellets were solubilized overnight in 0.3 ml of NCS Tissue Solubilizer (Amersham International) and counted for radioactivity in 5 ml of scintillation fluid [0.5% PPO (2,5-diphenyloxazole) in toluene]. All points were from experiments performed in triplicate. Protein in the samples was determined by a micro-Lowry method using

bovine serum albumin as standard (Bensadoun & Weinstein, 1976).

Photoaffinity labelling

Photoaffinity labelling of the receptor complex was performed by the method of Möhler *et al.* (1980). Purified receptor complex ($50\text{ }\mu\text{l}$; $B_{\text{max}} = 60\text{ pmol/ml}$ for [^3H]flunitrazepam) was incubated with 70 nM-[^3H]flunitrazepam in a total volume of $60\text{ }\mu\text{l}$ for 1 h at 0°C . It was then exposed to a u.v. light source (Phillips TL 40W/08 tube) at a distance of 20 cm for 10 min at 0°C . Flunitrazepam was then added to a final concentration of $30\text{ }\mu\text{M}$ and incubation continued for 1 h. A $60\text{ }\mu\text{l}$ aliquot of 24% (w/v) trichloroacetic acid was added, followed by incubation for 30 min at 0°C and centrifugation at $11\,000\text{ g}$ for 10 min. The pellet was washed twice with acetone (250 μl) and subjected to SDS/polyacrylamide-gel electrophoresis as described below. A control experiment included flunitrazepam ($30\text{ }\mu\text{M}$) before addition of [^3H]flunitrazepam. After SDS/polyacrylamide-gel electrophoresis, gels were treated with PPO (Skinner & Griswold, 1983), dried and subjected to fluorography at -70°C on Fuji RX film for 7 days.

SDS/polyacrylamide-gel electrophoresis

Samples were concentrated by precipitation in 12% (w/v) trichloroacetic acid and washed twice with acetone. Precipitated protein was dissolved in sample buffer containing 60 mM-Tris/HCl, pH 6.8, 2% (w/v) SDS, 10% (w/v) glycerol, 10 mM-dithiothreitol and 0.01% (w/v) Bromophenol Blue, and heated for 2 min at 100°C . SDS/polyacrylamide-gel electrophoresis was performed with 1 mm-thick gels by using the system of Laemmli (1970) with a 7–17%-(w/v)-polyacrylamide gradient. Gels were silver-stained by a modification of the method of Morrissey (1981) as described by Sigel & Barnard (1984).

RESULTS

Purification of the GABA/benzodiazepine-receptor complex

The GABA/benzodiazepine-receptor complex was purified from a Triton X-100 extract of crude synaptic membranes of pig cerebral cortex by affinity and ion-exchange chromatography. Table 1 lists the results from a typical purification. [^3H]Flunitrazepam-binding activity was purified 2200-fold with a yield of 2%. The greatest loss of binding activity, as indicated by the yield of [^3H]muscimol binding activity (20%), occurred during the affinity-chromatography step. Less than 15% of the applied binding activities were detected in the run-through and wash fractions, indicating that the low yield results from either receptor inactivation or chlorazepate-resistant binding of the receptor to the affinity column.

Binding characteristics of the Triton X-100-purified receptor complex

The characteristics of [^3H]muscimol and [^3H]flunitrazepam binding to the receptor complex purified in Triton X-100 medium are shown in Fig. 1. The binding of both ligands was saturable, and Scatchard analysis of the binding curves described a single high-affinity binding site in each case. The dissociation constants (K_D) for [^3H]muscimol and [^3H]flunitrazepam

Table 1. Purification of the Triton X-100-solubilized GABA/benzodiazepine-receptor complex

Binding was determined by a precipitation/centrifugation assay as described in the Experimental section by using 40 nM-[3 H]muscimol and 30 nM-[3 H]flunitrazepam. Results are representative of ten individual preparations.

Fraction	Protein (mg)	Specific binding activity (pmol/mg)		Yield of [3 H]flunitrazepam binding (%)
		[3 H]Muscimol	[3 H]Flunitrazepam	
Homogenate	13800	0.05*	0.44	100
Crude synaptic membranes	1980	0.36*	1.32	43
Soluble receptor	1380	2.02†	0.94†	21
Affinity-column eluate	0.185	3270	—‡	—‡
DEAE-cellulose column eluate	0.125	2580	960	2

* Determination underestimated owing to presence of endogenous GABA.

† Before binding assay, soluble receptor (0.5 ml) was dialysed against 20 mM-potassium phosphate (pH 7.4)/0.1 mM-EDTA/0.5% Triton X-100/0.02% NaN_3 (500 ml).

‡ Binding was not determined owing to the presence of chlorazepate.

Fig. 1. Binding of [3 H]muscimol and [3 H]flunitrazepam to the Triton X-100-purified GABA/benzodiazepine-receptor complex

[3 H]Muscimol (●) and [3 H]flunitrazepam (○) binding was measured over a concentration range from 0.5 to 60 nM-[3 H]ligand by using 50 μ M-muscimol or 30 μ M-flunitrazepam respectively at each point to determine background. Results are typical for three separate experiments.

binding were 14 ± 3 nM and 14 ± 2 nM respectively. The ratio of [3 H]muscimol to [3 H]flunitrazepam binding sites was in the range 2.2–2.8, with maximum specific binding activities ($B_{\max}$) of 3.5 and 1.4 nmol/mg of protein respectively. The dissociation constants and ratio of $B_{\max}$ values describing binding to the purified and crude solubilized receptor complex were similar (Table 2), indicating no selective inactivation of either binding site during the purification procedure.

Secobarbital (1 mM) and propanidid (1 mM) had no effect on the binding of 10 nM-[3 H]muscimol to the purified receptor complex. In addition, benzodiazepine binding at 1 nM-[3 H]flunitrazepam was unaffected by the addition of secobarbital (1 mM) or GABA (0.1 mM).

Polypeptide composition and photoaffinity labelling of the purified receptor

SDS/polyacrylamide-gel electrophoresis of the purified receptor showed the existence of two major bands with M_r values 49000 and 55000 (Fig. 2a). When labelled irreversibly with [3 H]flunitrazepam after u.v. illumination (Fig. 2c), radioactivity was incorporated predominantly into the M_r -49000 polypeptide, although some radioactivity was also detectable in the M_r -55000 band. The receptor complex purified from cerebellum displayed a similar polypeptide pattern (Fig. 2b) to that derived from cortex and incorporated radioactivity predominantly into the M_r -49000 polypeptide (Fig. 2d). No radioactivity was incorporated into the M_r -55000 band, although traces were detected that correlated with a faint band at M_r 43000. Although the extent of incorporation into the M_r -43000 band varied slightly between individual preparations of cerebellar receptor, radioactivity was never detected in the polypeptide of M_r 55000, even after long exposure times. The M_r -55000 band from cerebellum was particularly diffuse, and may represent a doublet. Protein and radioactivity detected at the top of the gels indicate a degree of receptor aggregation. The receptor complex was not labelled in the presence of unlabelled 30 μ M-flunitrazepam (Fig. 2e).

Purification of the receptor complex in the presence of Chaps

Solubilization of GABA-receptor binding activity which retained optimal sensitivity to stimulation by

Table 2. Binding properties of the Triton X-100 purified GABA/benzodiazepine-receptor complex

Binding of ligands to the crude solubilized receptor was measured after dialysis as described in the footnote to Table 1. Binding coefficients were determined from Scatchard plots, with experimental details as described in the legend to Fig. 1. Values of K_D and the ratio of B_{max} values are the means for three independent preparations.

Fraction	Ligand	K_D (nM)	B_{max} (pmol/mg of protein)	Ratio of B_{max} values (muscimol/flunitrazepam)
Crude solubilized receptor	Muscimol	13	2.7	2.1
	Flunitrazepam	11	1.3	
Purified receptor	Muscimol	14	3500	2.5
	Flunitrazepam	14	1400	

barbiturates and the anaesthetic propanidid was found after treatment of membranes with low concentrations of Chaps [the preceding paper (Kirkness & Turner, 1985)]. The GABA/benzodiazepine receptor was purified essentially as before, but with 5 mM-Chaps replacing Triton X-100 in all stages. The yield of purified receptor protein obtained by this procedure was reduced to 25% of that obtained before (Table 3). This was principally due to the low solubilizing power of 5 mM-Chaps. When membranes were solubilized with 25 mM-Chaps/0.2% L- α -lecithin and subsequently purified in the presence of 5 mM-Chaps/0.05% L- α -lecithin, the yield increased to between 50 and 60% of that obtained by extraction and purification in the presence of Triton X-100.

Table 3 also shows differences in the characteristics of ligand binding to the receptor complex extracted by the two detergents. At the concentration of ligand examined, the ratio of [3 H]muscimol to [3 H]flunitrazepam specific binding activity was in the range 1.9–2.8 (ten preparations) after extraction with Triton X-100, and 0.3–0.6 (four preparations) after extraction with Chaps. Similar ratios were found after purification of the receptor complex. This decreased ratio, after extraction with Chaps, resulted principally from a substantial decrease in [3 H]muscimol specific binding activity.

Stimulation of ligand binding to the Chaps-purified receptor complex

As shown in Fig. 3, propanidid caused a concentration-dependent stimulation of [3 H]muscimol binding to the Chaps-purified receptor complex. Binding was stimulated to a maximum of 138% of control binding at the highest concentration of propanidid examined (5 mM).

Secobarbital at 1 mM also caused a stimulation of [3 H]muscimol binding to the Chaps-purified preparation (128%). In addition, the binding of 1 nM-[3 H]flunitrazepam to this preparation was stimulated by 1 mM-secobarbital (118%) and 0.1 mM-GABA (125%). Assay conditions were as described in the legend to Fig. 3. The extent to which binding to the purified receptor was stimulated was not affected by the concentration of Chaps (5 or 25 mM) used to extract the receptor from the membrane.

In agreement with a previous report (Sigel & Barnard, 1984), we found that SDS/polyacrylamide-gel electrophoresis of the Chaps-purified receptor produced a polypeptide pattern similar to that isolated in media containing Triton X-100, with only two major bands, at M_r 49000 and 55000 (results not shown).

Fig. 2. Photoaffinity labelling and SDS/polyacrylamide gel electrophoresis of the Triton X-100 purified GABA/benzodiazepine-receptor complex from cortex and cerebellum

Purified receptor (3 pmol of [3 H]flunitrazepam binding sites) from (a) cortex and (b) cerebellum was subjected to SDS/polyacrylamide-gel electrophoresis and silver-stained for protein. Purified receptor (3 pmol of [3 H]flunitrazepam binding sites) from (c) cortex and (d) cerebellum was photoaffinity labelled and subjected to SDS/polyacrylamide-gel electrophoresis and fluorography as described in the Experimental section. In (e), purified receptor from cortex was photoaffinity-labelled in the presence of unlabelled flunitrazepam.

DISCUSSION

Solubilization of membrane-bound receptors is a major problem associated with their purification, as the

Table 3. Solubilization and purification of the GABA/benzodiazepine receptor in media containing Triton X-100 or Chaps

Specific binding to the receptor complex was measured as described in Table 1, and data for individual isolation procedures are representative for two to ten independent preparations.

Solubilizing agent	Fraction	Protein (mg)	Specific binding (pmol/mg)		Ratio $\frac{[^3\text{H}]\text{muscimol}}{[^3\text{H}]\text{flunitrazepam}}$
			[^3H]Muscimol	[^3H]Flunitrazepam	
1. 2% Triton X-100	Solubilized DEAE-cellulose-column eluate	1380	2.02*	0.94*	2.15
		0.125	2580	960	2.69
2. 5 mM-Chaps	Solubilized DEAE-cellulose-column eluate	535	0.37†	0.88†	0.42
		0.031	153	435	0.35
3. 25 mM-Chaps/ 0.2% L- α -lecithin	Solubilized DEAE-cellulose-column eluate	1156	0.51‡	0.97‡	0.53
		0.070	283	676	0.42

* This determination was made after dialysis of soluble receptor as in Table 1.
† As *, but substituting 5 mM-Chaps for 0.5% (w/v) Triton X-100 in dialysis buffer.
‡ As *, but substituting 5 mM-Chaps/0.05% L- α -lecithin for 0.5% (w/v) Triton X-100 in dialysis buffer.

Fig. 3. Enhancement by propanidid of [^3H]muscimol binding to the Chaps-purified receptor complex

Specific binding of 10 nM-[^3H]muscimol to a receptor preparation solubilized and purified in the presence of 5 mM-Chaps was measured in the absence and presence of a range of propanidid concentrations. Each point represents the mean of determinations from two independent preparations.

procedure can often affect the stability and ligand-binding characteristics of the proteins involved. We have examined properties of the GABA/benzodiazepine receptor from pig cerebral cortex after purification from Triton X-100 and Chaps detergent extracts. Differential solubilization of [^3H]flunitrazepam- and [^3H]muscimol-binding activities (Stephenson *et al.*, 1982) and of two types of benzodiazepine receptor (Lo *et al.*, 1982) has been reported after treatment of brain membranes with Triton X-100. These properties could explain the differences in ligand-binding characteristics and subunit composition reported for the GABA/benzodiazepine-receptor complex isolated from Triton X-100 extracts (Martini *et al.*, 1983) and from sodium deoxycholate extracts (Sigel *et al.*, 1983). In the present study, [^3H]flunitrazepam- and

[^3H]muscimol-binding activities solubilized with 2% Triton X-100 were found to co-purify during affinity chromatography and DEAE-cellulose chromatography. The benzodiazepine-binding activity was enriched 2200-fold, with a maximum specific binding activity of 1.4 nmol/mg of protein. The purification procedure caused little change in the affinity of ligand binding, and the ratio of [^3H]muscimol to [^3H]flunitrazepam binding sites in the purified preparation was similar to that observed in the crude soluble extracts (Table 2; Lo *et al.*, 1983). Martini *et al.* (1983) reported a ratio of [^3H]muscimol to [^3H]flunitrazepam binding sites of about 1 after isolation of the receptor in Triton X-100. We have observed a ratio (2.2–2.8) more consistent with that reported by Sigel *et al.* (1983) after purification in Triton X-100 from a sodium deoxycholate extract (3.5–3.8). This higher value may result from the greater instability of benzodiazepine binding in the presence of sodium deoxycholate (Stephenson *et al.*, 1982). The receptor complex from rat brain extracted and isolated in Triton X-100 was reported to contain only one subunit, of M_r 60000 (Martini *et al.*, 1983). More recent reports suggest that the receptor is comprised of two polypeptides, of M_r 53000 and 57000 (bovine and rat brain; Sigel *et al.*, 1983; Sigel & Barnard, 1984) or M_r 48500 and 54500 (rat brain; Taguchi & Kuriyama, 1984). We have also observed two major bands (M_r 49000 and 55000) after SDS/polyacrylamide-gel electrophoresis of the receptor purified from pig cortex or cerebellum. The M_r -55000 band appeared slightly diffuse and may represent a doublet. Photoaffinity labelling of the receptor complex purified from cortex resulted in predominant incorporation of radioactivity into the M_r -49000 band, although some radioactivity was also detectable in the M_r -55000 polypeptide. A similar distribution was reported after labelling of the receptor purified from bovine cortex (Sigel *et al.*, 1983). In contrast, no radioactivity was incorporated into the M_r -55000 band from cerebellum, although traces were detected at M_r -43000, corresponding to a band that stained weakly for protein (Fig. 2d). Although this difference in labelling may result from differential proteolytic action on the receptor preparations from the

two brain regions, it is consistent with several lines of evidence suggesting heterogeneity in central-type benzodiazepine receptors. The triazolopyridazine CL218872 can interact with benzodiazepine receptors in a number of brain regions, in a manner suggesting receptor heterogeneity or negative cooperativity (Squires *et al.*, 1979; Klepner *et al.*, 1979). From these studies, multiple benzodiazepine receptors were predicted in cortex and hippocampus, but not cerebellum. Corresponding results have been obtained from experiments employing photo-affinity labelling of membrane-bound benzodiazepine receptors. By using membrane preparations from cerebellum, [³H]flunitrazepam was irreversibly incorporated into a single polypeptide (M_r 51 000), whereas in other brain areas, additional polypeptides (M_r 53 000, 55 000 and 57 000) were also labelled (Sieghart & Karobath, 1980; Sieghart & Drexler, 1983). We have shown that this characteristic is retained in purified preparations of receptor. The difference in labelling of the purified receptor from cortex and cerebellum may result from heterogeneity in the M_r -55 000 subunits. Alternatively, regional differences in receptor conformation may permit non-specific labelling of the M_r -55 000 band in cortex but not in cerebellum.

The ability of barbiturates and propanidid to stimulate solubilized [³H]muscimol-binding activity was found to be optimal after extraction from membranes with low concentrations of Chaps (Kirkness & Turner, 1986). [³H]Muscimol binding to the GABA/benzodiazepine receptor extracted and purified in the presence of 5 mM-Chaps retained its sensitivity to these anaesthetic agents. The extent of maximum enhancement is relatively low (135–140%) compared with the effect in crude solubilized and membrane preparations (Kirkness & Turner, 1986), indicating partial inactivation of the effect during isolation. In agreement with Sigel & Barnard (1984), [³H]flunitrazepam binding to a Chaps-purified receptor preparation was also weakly stimulated by secobarbital and GABA. Increasing the concentration of Chaps used to extract the receptor from the membranes to 25 mM approximately doubled the yield of purified receptor, but did not affect the ability of secobarbital and propanidid to enhance ligand binding. The relatively rapid exchange of 25 mM-Chaps for 5 mM-Chaps in the affinity column wash may prevent the apparent inhibitory effect of high Chaps concentrations on barbiturate sensitivity (Kirkness & Turner, 1986).

A comparison of receptor preparations isolated in Triton X-100 or Chaps shows that the ratio of [³H]muscimol to [³H]flunitrazepam binding was much reduced after purification with Chaps. Although the specific binding of both ligands was lower, [³H]muscimol binding was reduced to a much greater extent (Table 3). The Chaps-purified preparation displayed no differences in polypeptide composition from that of the preparation isolated in Triton X-100, indicating that the differences in [³H]muscimol binding and responsiveness to barbiturates (and propanidid) may result from differences in receptor conformation after isolation by the different procedures. Treatment of membranes with low concentrations of Triton X-100 has been shown to cause considerable activation of GABA receptor binding (Enna & Snyder, 1977; Chiu & Rosenberg, 1979). In addition, studies with the purified receptor indicate that activation is caused by conversion of [³H]muscimol binding sites from low to high affinity (Sigel & Barnard, 1984). Triton X-100 binds

very tightly to proteins and may displace receptor-bound phospholipids that could play an important role in the interactions between the recognition sites of the receptor complex. A possible involvement of lipid components in the interactions is suggested by the observation that the potency of a series of barbiturates to enhance GABA (Whittle & Turner, 1982; Olsen & Snowman, 1982) and benzodiazepine (Leeb-Lundberg & Olsen, 1982) binding to membranes correlates well with their lipid solubility. Propanidid is also highly-lipid-soluble. The stereospecificity of the interaction of some barbiturates with receptor binding would, however, suggest that protein interactions may also be involved (Olsen & Snowman, 1982).

By using a Chaps-purified receptor preparation, in which interactions between the various recognition sites can be demonstrated, it may be possible to obtain a better understanding of the molecular interactions underlying regulation at the GABA synapse.

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