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Monoclonal antibodies reveal structural homogeneity of γ-aminobutyric acid/benzodiazepine receptors in different brain areas

(subunits/epitopes/species specificity)

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ABSTRACT **Monoclonal antibodies (mAb) against a γ-aminobutyric acid/benzodiazepine receptor complex ($GABA_A$/BZR) were produced by using spleen cells from a mouse immunized with $GABA_A$/BZR purified from bovine cerebral cortex. The mAb, most of which were of the IgG1 isotype could be divided into four groups (I–IV) specifying different antigenic structures. On immunoblots, group I mAb recognized exclusively the M_r 55,000 β-subunit, while groups II and IV mAb recognized the M_r 50,000 α-subunit of bovine $GABA_A$/BZR. Three of the four groups of mAb (I, III, and IV) crossreacted with both human and rat $GABA_A$/BZR with the same subunit specificity as in bovine brain; the fourth group (II) crossreacted with human but not with the rat receptor. The binding sites for benzodiazepines as well as the high and low affinity GABA sites reside on the same structural complex as shown by immunoprecipitation. Ligand binding to these sites was not inhibited by mAb. Since quantitative immunoprecipitation of $GABA_A$/BZR was achieved with mAb selective for either the α- or β-subunit, both subunits occur in each individual receptor complex. The pattern of immunoblot staining suggests that the smaller α-subunit is not a processing product of the larger β-subunit. Both α- and β-subunits were present in all brain areas and species tested (rat cerebral cortex, cerebellum, and hippocampus; bovine cerebral cortex and cerebellum; human cerebral cortex). This suggests a uniform subunit composition of the receptor throughout the brain in contrast to earlier evidence for a heterogeneous subunit composition based on photoaffinity labeling.**

The major inhibitory neurotransmitter in the brain is γ-aminobutyric acid (GABA), which exerts its effects mainly through a $GABA_A$ receptor complex localized in the subsynaptic membrane of the target neuron. This receptor complex is thought to consist of the $GABA_A$ receptor, a chloride ionophore and in most, if not all, cases a regulatory unit, the benzodiazepine receptor (BZR) (1). Depending on the type of BZR ligand, $GABA_A$ receptor function can be either enhanced or reduced. Thereby the degree of anxiety, the extent of muscle tension, the state of vigilance, and the likelihood of convulsions can be either enhanced or reduced (1).

The protein constituents of the receptor complex were previously identified by photoaffinity labeling of benzodiazepine binding sites in rat brain membranes. With this technique, a protein component of M_r 50,000 (α-subunit) was found in all brain regions (2–6), while in some brain areas, an additional protein of M_r 55,000 (β-subunit) was also labeled (4–6). Thus, a receptor heterogeneity appeared to exist in the brain, which seemed to provide a framework to explain differences in the pharmacological spectrum of BZR ligands (7, 8).

To further investigate a possible heterogeneity of the $GABA_A$/benzodiazepine receptor complex ($GABA_A$/BZR), monoclonal antibodies (mAb) were raised against a $GABA_A$/BZR preparation purified from bovine cerebral cortex (9–11). In this report, we describe the properties of a collection of 16 mAb to $GABA_A$/BZR: The mAb (*i*) precipitated a $GABA_A$/BZR complex, (*ii*) recognized four different antigenic sites on the receptor, (*iii*) showed subunit specificity, and (*iv*) crossreacted with rat and human $GABA_A$/BZR. Thus, these mAb provide additional tools for the structural analysis of $GABA_A$/BZR.

MATERIALS AND METHODS

Purification of $GABA_A$/BZR. $GABA_A$/BZR was isolated from bovine cerebral cortex by affinity purification on immobilized benzodiazepine (9–11). The receptor preparations contained 10–35 μg of protein per ml and 0.05% Triton X-100; they were concentrated ≈10-fold by ultrafiltration for immunization and immunoblotting. $GABA_A$/BZR preparations from postmortem human cerebral cortex, bovine cerebellum, and from rat whole brain or different rat brain regions were prepared as described for bovine cerebral cortex. The human postmortem brain material was obtained from the Institute of Pathology, Kantonsspital, Basel (Switzerland).

Hybridoma Production. Eight-week-old female BALB/cJ mice were immunized (12, 13) by both subcutaneous and intraperitoneal (i.p.) injections of 100 μg of bovine cerebral cortex $GABA_A$/BZR (0.5% Triton X-100) in complete Freund's adjuvant followed 3 weeks later by i.p. injection of 100 μg of antigen in incomplete Freund's adjuvant. The mouse with the highest specific antibody titer in serum was given an i.p. booster injection 9 weeks later on 2 successive days with 120 μg of antigen (≧0.5% Triton X-100) without adjuvant each time. Two days later, $\approx 70 \times 10^6$ spleen cells and 40×10^6 PAI myeloma cells (14) were fused as described elsewhere (12) and distributed into 240 2-ml tissue culture wells. All wells showed growth of hybridomas. Initial screening of the culture supernatants was performed in a solid-phase antibody binding assay (see below), using a $GABA_A$/BZR preparation from bovine cerebral cortex.

Purification and ^{125}I Labeling of mAb. mAb were affinity-purified from culture supernatant by using an anti-mouse Ig Sepharose-4B column (15). Purified mAb were labeled with ^{125}I by the chloramine-T method (16), without using Na metabisulfite, to a specific activity of 5–10 μCi per μg of mAb (1 Ci = 37 GBq).

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Abbreviations: mAb, monoclonal antibody(ies); GABA, γ-aminobutyric acid; $GABA_A$/BZR, $GABA_A$/benzodiazepine receptor complex.

Solid-Phase Antibody Binding Assay. Wells of polyvinylchloride microtiter plates were incubated overnight at room temperature (RT) with 50 μl of bovine, human, or rat $GABA_A$/BZR (2–5 μg of protein per ml) in P_i/NaCl (phosphate-buffered saline: 10 mM Na phosphate, pH 7.2/140 mM NaCl) followed by 3% bovine serum albumin for 1 hr. The coated wells were then incubated with 50 μl of hybridoma culture supernatant overnight at RT, followed by ^{125}I-labeled sheep anti-mouse Ig antibodies for 5 hr. After rinsing, the bound radioactivity was counted. For details of the procedure, see refs. 12, 13, and 17.

Isotype Determination of mAb. Plastic wells coated with bovine $GABA_A$/BZR were incubated with mAb (see solid-phase antibody binding assay), followed by incubation for 4 hr at RT with horseradish peroxidase-conjugated anti-mouse Ig isotype antibodies (Nordic, Lausanne, Switzerland and New England Nuclear). Binding of the latter antibodies was tested in a colorimetric enzyme assay containing 0.1 M potassium citrate (pH 5.5), 2.5 mM H_2O_2, and 2 mM 2,2'-azino-di-[3-ethylbenzthiazoline-sulfonate] (Boehringer) (15).

Competition Solid-Phase Antibody Binding Assay. Plastic wells coated with bovine $GABA_A$/BZR (see solid-phase antibody binding assay) were incubated over night at RT with 0.1 ml of mAb in a concentration (≧20 μg/ml) sufficient to saturate all corresponding antigenic determinants (blocking mAb). Then, 25 μl of the same or another ^{125}I-labeled mAb (200,000–600,000 cpm) was added to the first mAb solution and incubation was continued for 2 hr. After rinsing, the radioactivity bound in the wells was counted. Controls contained a mAb unrelated to $GABA_A$/BZR instead of the blocking mAb. For details of the procedure, see ref. 15.

Immunoprecipitation. One hundred microliters of a purified bovine $GABA_A$/BZR preparation (20–30 μg of protein per ml) and 200 μl of hybridoma culture supernatant were incubated in Eppendorf tubes for 2 hr at 4°C. After addition of 200 μl of a 50% goat anti-mouse IgG agarose slurry (Sigma, A-653) in P_i/NaCl containing 0.2% Triton X-100 and 1% bovine serum albumin, the tubes were rotated for 2.5 hr and centrifuged. Supernatants and pellets (washed once with 1 ml of cold P_i/NaCl/0.2% Triton X-100/1% bovine serum albumin) were separately assayed for radioligand binding sites with the benzodiazepine [^{3}H]flunitrazepam (10 nM, 60 min) and the $GABA_A$ receptor ligand [^{3}H]muscimol (100 nM, 30 min). In addition, [^{3}H]flunitrazepam binding was assayed in the presence of 10 μM GABA. Radioligand binding with 300 μl of supernatant was done in 1 ml (final volume) at 4°C followed by a polyethyleneglycol precipitation and filtration of radioligand–receptor complex on glass fiber filters as described (18). Radioligand binding to the pellet was performed in 1 ml of assay buffer (50 mM Tris citrate, pH 7.1/0.2% Triton X-100) at 4°C. Radioligand bound to agarose immune complex was collected on glass fiber filters and washed with cold buffer. Controls contained unrelated hybridoma culture supernatants. For each [^{3}H]flunitrazepam and [^{3}H]muscimol binding test, nonspecific binding was determined in parallel in the presence of a large excess of an unlabeled ligand (3 μM diazepam or 1 mM GABA, respectively).

Immunoblotting. Immunoblots were prepared essentially according to Towbin *et al.* (19). $GABA_A$/BZR (20–200 μg) was electrophoresed under reducing conditions on a $NaDodSO_4$/15% polyacrylamide gel. After electroblotting the proteins onto a nitrocellulose membrane sheet (Millipore, type HA, 0.45-μm pore size: 1 A for 90 min; transfer buffer, 25 mM Tris·HCl/190 mM glycine/20% (vol/vol) methanol/0.01% $NaDodSO_4$, pH 8.3), the protein binding sites on the nitrocellulose were saturated by incubation in 0.05% Tween 20 in P_i/NaCl (P_i/NaCl/Tween) three times for 10 min (20). Strips of the membrane were each incubated overnight at RT in 5 ml of undiluted hybridoma culture supernatant while being agitated. After three 10-min washes in P_i/NaCl/Tween, the strips were incubated for 3 hr with 5 ml of ^{125}I-labeled sheep anti-mouse Ig antibodies (10^6 cpm/ml) in P_i/NaCl/Tween, followed by three washes in P_i/NaCl/Tween and one wash in P_i/NaCl. The dried strips were taped on filter paper and autoradiographed. Molecular weight markers were routinely run in a slot of the same slab gel and, after transfer onto nitrocellulose, stained with 0.1% amido black (21).

RESULTS

In the initial screening with a solid-phase antibody binding assay 28 hybridoma cultures were identified, which produced mAb (bd-1 to bd-28) that showed binding to $GABA_A$/BZR preparations (7- to 40-fold background). Of these, 16 were selected for further analysis, based on the stability of the hybridomas and the antibody titer of the supernatants, and cloned. Twelve of the mAb were of γ1, two of γ2b, and two of μ heavy-chain isotype. All had κ light chains.

Antigen Specificity. Because of the occurrence of some impurities in the $GABA_A$/BZR preparations, the antigen specificity of the mAb had to be verified by immunoprecipitation. The mAb were expected to precipitate a protein complex containing the binding sites for benzodiazepines and GABA. Benzodiazepine binding sites were assayed by [^{3}H]-flunitrazepam binding, high-affinity GABA sites were measured by [^{3}H]muscimol binding, and low-affinity GABA sites were tested by the allosteric enhancement of [^{3}H]flunitrazepam binding in the presence of micromolar concentrations of GABA. In controls of immunoprecipitation, containing no mAb or an unrelated mAb, only ≈7% of the ligand binding activities of a bovine $GABA_A$/BZR preparation was found in the pellet (Table 1). However, the 16 mAb selected (see above) precipitated a large fraction of the binding sites for benzodiazepines as well as low- and high-affinity GABA sites (Table 1). Most remarkably, each mAb precipitated the radioligand binding sites for benzodiazepines and GABA to the same extent (Table 1), indicating that these sites reside on the same structural complex. Thus, all 16 mAb recognized bovine $GABA_A$/BZR. The precipitation of binding sites did not reach maximal levels in all cases, probably because of insufficient mAb concentration in the culture supernatants. The concentration required for quantitative receptor precipitation differed by a factor of up to 10 for different mAb, as shown in titration experiments with purified mAb (22).

None of the mAb interacted with the radioligand binding sites. Controls that were not subjected to immunoprecipitation contained the same amount of radioligand bound as that found in the immunoprecipitated pellet plus supernatant. The presence of mAb did not alter radioligand binding to the solubilized receptor. Furthermore, the intensity of the immunoreaction in rat or bovine brain sections was unaltered in the presence of 20 μM diazepam or 5 mM GABA, as tested for mAb of groups I, II, and III (bd-17, bd-24, and bd-8, respectively) (J. G. Richards, personal communication).

Immunoprecipitation of $GABA_A$/BZR was also performed with a receptor preparation to which [^{3}H]flunitrazepam had been covalently attached by photoaffinity labeling (10 nM; 5 min of UV light; see ref. 2). When this preparation was subjected to immunoprecipitation with the 16 culture supernatants, the same percentage of radioactivity was recovered in the pellet as in the experiments described above, where the reversible radioligand binding assays were used for monitoring $GABA_A$/BZR.

Number of Epitopes. To test whether all 16 mAb interacted with the same or different sites on bovine $GABA_A$/BZR, it was determined if binding of one mAb to $GABA_A$/BZR could be inhibited by another mAb. From a large number of pairs of mAb tested in the competition solid-phase antibody

Table 1. Immunoprecipitation of radioligand binding sites of $GABA_A$/BZR preparations

mAb		Radioligand bound in pellet, % of total*	
Group†	Number	[^{3}H]Flunitrazepam‡	[^{3}H]Muscimol
I	bd-3	49	46
	bd-4	88	82
	bd-6	88	87
	bd-11	61	52
	bd-17	81	68
	bd-21	29	23
II	bd-9	92	85
	bd-14	95	96
	bd-23	93	90
	bd-24	96	77
	bd-26	93	95
III	bd-8	95	96
	bd-10	92	99
	bd-15	77	77
	bd-25	90	76
IV	bd-28	49	41
Unrelated mAb		7	6

*Total radioligand binding (100%) is the sum of binding in the pellet plus supernatant; 100% corresponds to controls (containing culture medium) without immunoprecipitation. The percentage of binding sites recovered in the pellet varied because the mAb concentration in the hybridoma culture supernatants used was not saturating in every case (see text). The results given (mean values of two experiments) refer to experiments with bovine $GABA_A$/BZR (cerebral cortex). Immunoprecipitation of $GABA_A$/BZR from rat whole brain yielded similar results except for group II mAb, which showed only background levels of binding in the pellet. $GABA_A$/BZR from human cerebral cortex could be immunoprecipitated to a similar extent as bovine receptor as tested with bd-17, bd-23, and bd-8.

†Each group (I–IV) comprises mAb with common epitope specificity (see text).

‡In the presence of 10 μM GABA, [^{3}H]flunitrazepam binding was enhanced in all cases by 20–40%, indicating the presence of low-affinity GABA sites.

Table 3. Binding of mAb (bd-*n*) to $GABA_A$/BZR as tested in a solid-phase antibody binding assay

mAb		^{125}I-labeled anti-mouse Ig antibody bound, cpm × 10^2	
Group*	Number	Bovine receptor	Rat receptor
I	bd-3	92	95
	bd-4	75	79
	bd-6	80	84
	bd-11	59	71
	bd-17	92	98
	bd-21	81	82
II	bd-9	78	3
	bd-14	100	3
	bd-23	91	5
	bd-24	90	6
	bd-26	87	3
III	bd-8	14	40
	bd-10	37	44
	bd-15	21	33
	bd-25	18	30
IV	bd-28	88	83

Plastic wells coated with $GABA_A$/BZR from different species (bovine and human cerebral cortex, rat whole brain) were incubated with the mouse mAb (bd-*n*: undiluted hybridoma culture supernatant). The immunoreaction of bd-*n* with the antigen was monitored by the subsequent addition of ^{125}I-labeled sheep anti-mouse Ig antibody. The radioactivity retained in the wells was counted. The extent of the immunoreaction with a receptor preparation from human cerebral cortex corresponded to that of bovine brain. The results represent plateau values as determined by titration experiments with culture supernatant.

*Each group comprises mAb with common epitope specificity (see text).

binding assay, two unambiguously different groups of mAb could be defined (groups I and II) (Table 2). Binding of the ^{125}I-labeled mAb bd-6 and bd-9 was inhibited by >95% by two different groups of mAb. Although the remaining mAb (bd-8, -10, -15, -25, and -28) could not be clearly classified on the basis of competition experiments (Table 2), they were tentatively placed in a third and fourth group of mAb, respectively, on the basis of their different plateau values obtained in the solid-phase antibody binding assay (Table 3). Group III mAb had a significantly lower plateau value than the mAb in groups I, II, and IV. Differences in plateau values have been found for several protein antigens to be an epitope-specific characteristic (unpublished observations). Definite evidence for four groups of mAb was provided by immunoblotting experiments and the species specificity described below. For a summary, see Table 4.

Subunit Specificity of mAb. Since subunits of M_r 50,000 and M_r 55,000 are known constituents of $GABA_A$/BZR (2, 4, 5, 10, 11), mAb were expected to recognize these proteins in immunoblot experiments. Purified preparations of bovine $GABA_A$/BZR were separated by $NaDodSO_4$/polyacrylamide gel electrophoresis, electroblotted to nitrocellulose in the presence of 0.01% $NaDodSO_4$, and allowed to react with individual mAb (Fig. 1): group I mAb reacted with a protein of M_r 55,000 (β-subunit), groups II and IV mAb reacted with a protein of M_r 50,000 (α-subunit) (Table 4). Group III mAb did not react, presumably because the corresponding epitope was sensitive to the denaturing conditions (Fig. 1). Even when 0.1% of the zwitterionic detergent Empigen BB (23) was included in the electroblot buffer instead of 0.01% $NaDodSO_4$, group III mAb showed no response in immunoblot experiments. Alternatively, this epitope may be formed by both subunits. No additional immunoreactive proteins were observed when a crude soluble fraction of brain membranes was used instead of the purified receptor preparation, as tested with bd-17 and bd-24 (22). Furthermore, soluble fractions of peripheral tissues, such as bovine or rat

Table 2. Competition of mAb for binding to $GABA_A$/BZR as tested in a solid-phase antibody binding assay

^{125}I-labeled mAb	Inhibition of ^{125}I-labeled mAb by various nonradioactive mAb (bd-*n*)															
	Group I						Group II					Group III				Group IV
	bd-3	bd-4	bd-6	bd-11	bd-17	bd-21	bd-9	bd-14	bd-23	bd-14	bd-26	bd-8	bd-10	bd-15	bd-25	bd-28
bd-6	+	+	+	+	+	+	−	−	−	−	−	±	±	±	−	−
bd-9	±	−	−	−	±	−	+	+	+	+	+	−	−	−	−	−

Plastic wells coated with bovine $GABA_A$/BZR (cerebral cortex) were incubated with ^{125}I-labeled mAb in the presence of unlabeled mAb, which were in large excess over $GABA_A$/BZR. The radioactivity bound to the wells was counted. +, >95% inhibition; ±, 55–70% inhibition; −, <45% inhibition.

Table 4. Summary of classification of mAb and their species and subunit specificities

mAb		Subunit recognized in different species*	
Group†	Number‡	Human or bovine receptor	Rat receptor
I	bd-3, -4, -6§, -11¶, -17, -21¶	β-subunit	β-subunit
II	bd-9, -14, -23, -24, -26	α-subunit	NI‖
III	bd-8, -10, -15§, -25	ND**	ND**
IV	bd-28	α-subunit	α-subunit

*The results are based on immunoblots of $GABA_A$/BZR from rat cerebral cortex, hippocampus, and cerebellum; bovine cerebral cortex and cerebellum; and human cerebral cortex.
†Each group (I–IV) comprises mAb with common epitope specificity.
‡All mAb had κ light chains. The heavy-chain isotype was γ1 unless indicated otherwise.
§Heavy-chain isotype γ2b.
¶Heavy-chain isotype μ.
‖Group II mAb did not interact with rat $GABA_A$/BZR in either solid-phase antibody binding assay, immunoprecipitation, or immunoblotting. NI, no immunoresponse.
**Although group III mAb were reactive in immunoprecipitation (Table 1) and solid-phase antibody binding assay (Table 3), their subunit specificity could not be determined (ND) (see text).

kidney, liver, and heart showed no immunoreaction with either mAb (22). Thus, the mAb described are selective for the constituent proteins of $GABA_A$/BZR. At least, groups I, II, and IV mAb show subunit specificity.

Species Specificity of mAb. Benzodiazepine receptors occur in all higher vertebrates (24). We therefore tested whether the mAb raised against bovine $GABA_A$/BZR would crossreact with the receptor complex from human and rat brain. The reactivities of the mAb with a $GABA_A$/BZR preparation from human cerebral cortex were virtually identical to those with the bovine receptor, as shown by immunoprecipitation (Table 1), solid-phase antibody binding assay (Table 3), and immunoblot experiments (Table 4). Some of the reactivities with the rat receptor, however, differed from those with the bovine and human receptor. While mAb from groups I, III, and IV bound to the receptor from rat, bovine, and human brain, group II mAb interacted with the bovine and human receptor but not with the rat receptor. This was apparent by immunoprecipitation (Table 1), by solid-phase antibody binding assay (Table 3), and by immunoblot experiments (Figs. 1 and 2, Table 4). Thus, the epitope recognized on the α-subunit by group II mAb differs from that recognized on the same subunit by the group IV mAb. An additional site on $GABA_A$/BZR is recognized by group III mAb, as shown by its sensitivity to denaturation (Fig. 1) and the difference in plateau value in solid-phase antibody binding assay (Table 3). Thus, four different epitopes of $GABA_A$/BZR are recognized by the mAb described. Three of them are conserved among bovine, rat, and human brain.

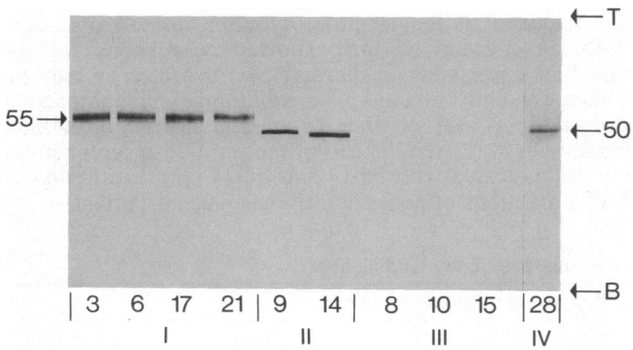

FIG. 1. Autoradiograph of an immunoblot with $GABA_A$/BZR purified from bovine cerebral cortex. mAb representing all four groups (I–IV) are indicated by the number (bd-*n*) at the bottom of each strip. The remaining six mAb were tested in another immunoblot experiment and showed the same group-specific reactivities (Table 4). Top (T) and bottom (B) of the gel as well as the positions of the M_r 55,000 and M_r 50,000 bands are indicated by arrows.

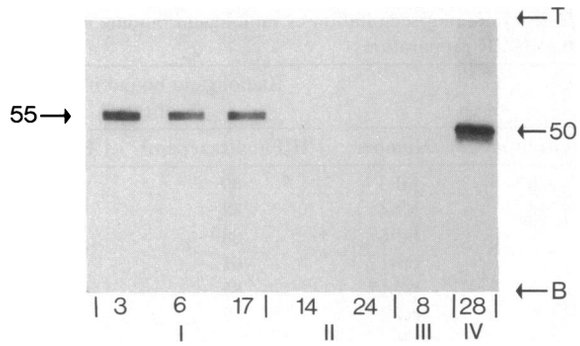

FIG. 2. Autoradiograph of an immunoblot with $GABA_A$/BZR purified from rat cerebellum. mAb representing all four groups (I–IV) are indicated by the number (bd-*n*) at the bottom of each strip. Top (T) and bottom (B) of the gel as well as the positions of the M_r 55,000 and M_r 50,000 bands are indicated by arrows.

Subunit Composition of $GABA_A$/BZR in Rat, Bovine, and Human Brain. With two types of benzodiazepine photoaffinity markers, the α-subunit was previously identified in all species and brain regions tested (2–6, 10, 11). In contrast, a β-subunit could be clearly detected by photolabeling only in rat hippocampus, while no trace of a β-subunit was found, for example, in rat cerebellum (4, 5). Thus, a heterogeneous receptor structure appeared to exist. However, by using the subunit-specific mAb in immunoblot experiments with $GABA_A$/BZR from rat cerebellum, the α-subunit as well as the β-subunit was detected (Fig. 2). Likewise, both subunits were found by immunoblotting of $GABA_A$/BZR preparations from rat hippocampus and cerebral cortex. Thus, the immunological experiments suggest a similar subunit composition throughout the rat brain. Both subunits could also be identified immunologically in bovine and human brain—e.g., in bovine cerebellum and cerebral cortex (Fig. 1) and in human cerebral cortex (Table 4).

DISCUSSION

The 16 mAb described are directed against the $GABA_A$/BZR complex in the brain, as shown by immunoprecipitation (Table 1) and immunoblotting (Figs. 1 and 2, Table 4). There was no immunoreaction in peripheral organs such as kidney, liver, and heart, which contain high levels of the peripheral type of benzodiazepine binding site, as tested by immunoblotting and immunohistochemistry (22). This indicates that the mAb do not bind to structures containing the peripheral type of benzodiazepine binding site.

The mAb recognize four epitopes or epitopic regions on $GABA_A$/BZR (Table 4), as established by a combination of criteria defined by competition solid-phase antibody binding assay (Table 2), immunoblot experiments (Figs. 1 and 2), and species specificity (Tables 3 and 4). At least three of the four epitopes are subunit specific, two being located on the α-subunit (M_r, 50,000) and one on the β-subunit (M_r, 55,000) (Table 4).

At least two different genetic units seem to code for $GABA_A$/BZR, as indicated by the structural differences between the α-

and β-subunit. Although of similar molecular size, there was not sufficient homology between the two subunits for crossreactivity of the mAb in at least three antigenic regions (Figs. 1 and 2, Table 4). A precursor–product relationship for the two subunits seems to be excluded on the basis of mAb reactivity in immunoblots (Figs. 1 and 2). Furthermore, both subunits are known to differ in their ligand binding sites. Photoactivated benzodiazepine analogues are almost exclusively linked to the α-subunit and (apart from rat hippocampus) minimally or not at all to the β-subunit (2–6, 10, 11).

Since mAb of different subunit specificity immunoprecipitated the entire population of binding sites present in a receptor preparation (Table 1), a $GABA_A$/BZR consists of a mixture of α- and β-subunits. These subunits contain the binding sites for benzodiazepines as well as the high- and low-affinity sites for GABA, since these sites were coprecipitated (Table 1).

The two subunits could be identified in the brain of all species tested (rat, cow, human: Table 4). Furthermore, within one species, both subunits were present in all brain areas tested, suggesting a uniform subunit composition of $GABA_A$/BZR throughout the brain. This conclusion is supported by histological evidence. Subunit-specific mAb revealed the same pattern of immunoreactivity in tissue sections comprising a wide variety of regions in either rat, bovine, or human brain (22). These results are in contrast to earlier findings with photoreactive benzodiazepines showing a regionally different subunit composition in rat brain. The α-subunit was identified throughout the rat brain (2–6), while a β-subunit could be clearly detected only in rat hippocampus and not, for example, in cerebellum (4–6). This finding gave rise to the hypothesis that a structural heterogeneity of $GABA_A$/BZR in the brain may be relevant for differences in pharmacological profile of benzodiazepine receptor ligands (7, 8). However, our immunological experiments clearly show the presence of both α- and β-subunits in all brain regions. No heterogeneity of $GABA_A$/BZR could be detected. Thus, differences in the pharmacological spectrum of benzodiazepine receptor ligands are not due to a heterogeneity in the subunit composition of $GABA_A$/BZR in the brain. It remains to be clarified why the regional pattern of photoaffinity labeling is not homogeneous.

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