Localization of the GABA_A Receptor in the Rat Brain with a Monoclonal Antibody to the 57,000 M_r Peptide of the GABA_A Receptor/Benzodiazepine Receptor/Cl⁻ Channel Complex

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The mAb 62-3G1 to the GABA_A receptor/benzodiazepine receptor/Cl⁻ channel complex was used with light-microscopy immunocytochemistry for studying the localization of the GABA_A receptors (GABA_R) in the rat brain. The results have shown a receptor distribution identical to the one obtained by others using ³H-muscimol binding in combination with autoradiographic techniques. The external plexiform layer of the olfactory bulb, cerebral cortex, granule cell layer of the cerebellum, hippocampus, dentate gyrus, substantia nigra, dorsolateral and medium geniculate nuclei, and the lateral posterior thalamic nucleus, among other areas, were rich in GABA_A receptor immunoreactivity. In the cerebellum the granule cell layer had more immunoreactivity than did the molecular layer. In the hippocampus the receptor was most abundant in the stratum oriens and in the molecular layer of the dentate gyrus. The immunocytochemical techniques have also allowed us to study the distribution of the GABA_A receptor with high-resolution light microscopy. These studies have shown that the GABA_A receptors are localized in neuronal membranes and concentrated in structures rich in GABAergic synapses, such as the cerebellar and olfactory glomeruli and the external plexiform layer of the olfactory bulb, the deep cerebellar nuclei, and the substantia nigra.

The mAb 62-3G1 was generated by immunizing mice with the affinity-purified GABA_A receptor/benzodiazepine receptor (BZDR) complex. This mAb bound to the 57,000 M_r peptide but not to the benzodiazepine binding 51,000 M_r peptide. The distribution of the GABA_R immunoreactivity in the rat brain colocalized better with ³H-muscimol than with ³H-benzodiazepine binding. Therefore, it is suggested that (1) the 57,000 M_r peptide that is recognized by the mAb 62-3G1 is the muscimol (GABA_A receptor agonist) binding subunit of the receptor complex, (2) there is an important population of brain GABA_A receptors that is not functionally coupled to the benzodiazepine receptors, and (3) both the BZDR-coupled and uncoupled forms of the GABA_A receptor are immunologically similar, if not identical.

The benzodiazepine receptor (BZDR) is a membrane protein associated with the GABA_A receptor, the Cl⁻ channel, and a receptor for barbiturates. Together, they form a membrane protein receptor complex (for reviews, see Ticku, 1983; Turner and Whittle, 1983; Richards and Möhler, 1984; Squires, 1984; Tallman and Gallager, 1985; Olsen et al., 1986). The receptor complex has been solubilized with various detergents and purified by affinity chromatography on immobilized benzodiazepines. The purified complex retains the binding activities for benzodiazepines, muscimol (GABA_A receptor agonist), and *t*-butylbicyclophosphorothionate (TBPS) (Sigel and Barnard, 1984; Kirkness and Turner, 1986). The TBPS, as well as picrotoxinin, is a ligand that binds to or very near to the Cl⁻ channel. The proteins of the complex interact functionally, probably by allosteric mechanisms, such that the binding of a ligand to its receptor alters the binding properties of the other receptor proteins for their respective ligands. Radioligand autoradiography techniques have been used for studying the topographical localization in the brain of the various receptor components of the complex. These studies have shown a good correlation for the localization of the benzodiazepine, bicuculline (used as a low-affinity GABA_A receptor ligand), and TBPS-binding activities (Unnerstall et al., 1981; Olsen et al., 1984; McCabe and Wamsley, 1986). It seems that the GABA_A receptor that forms part of the functional complex is the "low-affinity" receptor for GABA or muscimol (Bowery et al., 1984; Johnston, 1986; McCabe and Wamsley, 1986; Wamsley et al., 1986). There are also "high-affinity" GABA_A receptors associated with the Cl⁻ channel. The "high-affinity" GABA_A receptors might not be functionally coupled to the benzodiazepine receptors. This could explain, at least in part, the mismatching in the distribution of the ³H-muscimol and ³H-flunitrazepam (FNZ) binding in several brain regions (Palacios et al., 1980, 1981a; Unnerstall et al., 1981; Schoch et al., 1985; McCabe and Wamsley, 1986). Another complicating factor is the presence of both presynaptic (autoreceptors) and postsynaptic GABA_A and benzodiazepine receptors, as well as extrasynaptic receptors. Radioligand autoradiography is very useful for mapping receptors in the various regions of the tissue, but it lacks the resolution necessary for studying the precise cellular and subcellular localization of the receptors. The latter can be better accomplished by immunocytochemical techniques.

We have recently made monoclonal antibodies (mAbs) to the GABA_A receptor/benzodiazepine receptor complex. One of the antibodies (62-3G1) recognizes the 57,000 M_r peptide and it is exceptionally good for immunocytochemistry (see Vitorica et

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al., 1988, the following paper). In this paper we present the distribution of GABA_A receptor immunoreactivity in the rat brain.

Materials and Methods

Monoclonal antibodies and antisera. The anti-GABA receptor (GABAR) mAb 62-3G1 was obtained after immunizing BALB/c mice with the purified BZDR/GABAR complex from bovine brain, followed by fusion of the spleen cells with the myeloma line P3X63Ag8.6.5.3. The receptor complex was purified by affinity chromatography on the immobilized benzodiazepine Ro7-1986/1, followed by DEAE-Sephacel ion-exchange chromatography, as described elsewhere (see Vitorica et al., 1988, the following paper). The purified receptor showed, in SDS-PAGE, 2 main peptides of 51,000 and 57,000 *M_r*. The 51,000 *M_r* peptide could be photoaffinity-labeled with the benzodiazepines ³H-FNZ and ³H-Ro15-4513. In contrast, the 57,000 *M_r* peptide could be photoaffinity-labeled with ³H-muscimol. The mAb 62-3G1 (1) precipitated both the ³H-muscimol- and ³H-FNZ-binding activities of the affinity-purified receptor complex; (2) reacted with the immobilized purified receptor complex in solid-phase RIA assays, and (3) recognized the 57,000 *M_r* peptide but not the 51,000 *M_r* subunit in immunoblots.

The mAbs 6-4H2 and 8-6A2 were made following immunization of BALB/c mice with synaptosomal membranes. The making and characterization of these antibodies have been described elsewhere (De Blas et al., 1984).

The anti-rat brain glutamic acid decarboxylase (GAD) raised in sheep and the rabbit anti-GABA antisera were the generous gifts of Drs. Irwin J. Kopin and Robert Wenthold, respectively, from the NIH (Bethesda).

Peroxidase immunocytochemistry. This was based on our standard procedure (De Blas, 1984; De Blas et al., 1984). Sprague-Dawley rats, 35 d old, were perfused with either periodate/lysine/4% paraformaldehyde (PLP; McLean and Nakane, 1974) or 4% paraformaldehyde/0.1% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4 (4% paraformaldehyde/0.1% glutaraldehyde). Fixed and frozen brains were sliced (25 μm thick) with a microtome. The brain slices that were fixed with glutaraldehyde were incubated with 0.1 M lysine in 0.1 M phosphate buffer, pH 7.4, and 0.1% BSA for 1 hr prior to the immunoreaction.

All the slices were incubated for 16 hr at 4°C with the antibodies containing 0.3% Triton X-100. The antibody binding to the tissue was revealed by reaction with a biotin-labeled antibody, followed by an avidin-biotin-horseradish peroxidase complex (ABC procedure; Vector Labs) or (when indicated) by a peroxidase-antiperoxidase (PAP) technique. The displacement of 62-3G1 immunoreactivity by previous incubation of the antibody with 9 μg of affinity-purified GABAR/BZDR complex was used as a control. The conditioned medium of the parental myeloma P3X63Ag8.6.5.3. was also used as mAb control. Controls showed no immunocytochemical staining. Sheep and rabbit preimmune sera were used for antisera controls. The antibodies were used at the following dilutions, except where otherwise indicated: the hybridoma culture media containing the mAbs 62-3G1, 8-6A2, and 6-4H2 at 100×, 5× and no dilution, respectively; anti-GAD, anti-GABA and anti-NFP sera at 5000–10,000× dilutions.

Results

Figure 1 shows the distribution in the rat brain of the GABA_A receptor immunoreactivity with the anti-GABAR mAb 62-3G1. The immunoreactivity is highest in the areas with the highest concentration of high-affinity GABA_A receptor, as determined by ³H-muscimol binding. These are the granule cell layer of the cerebellum, the cerebral cortex, the hippocampus and dentate gyrus, the dorsolateral and medial geniculate nuclei, and the substantia nigra, among others. These areas are also known to have high contents of GABA and GAD. It is also worth noticing the low GABAR immunoreactivity in the corpus callosum, as well as in other areas rich in white matter. The immunoreactivity in all areas could be blocked by incubating the antibody with 9 μg of affinity-purified GABAR/BZDR complex from the bovine brain cerebral cortex (Figs. 2*B*, 8*B*).

Cerebellum

Figure 2*A* shows that the GABAR immunoreactivity concentrates at the granular cell layer. It is also present in the molecular layer and absent from the Purkinje cell layer (Fig. 3*A*) and from the white matter. This distribution agrees with the distribution of ³H-muscimol binding (Palacios et al., 1980, 1981a; McCabe and Wamsley, 1986). At higher magnification, it could be shown that, although granule cells have immunoreactivity in their membranes, the darkest reaction was on the cerebellar glomeruli (Fig. 3*D*). The glomeruli are also rich in GAD and GABA immunoreactivity (Fig. 2*C*; Saito et al., 1974; Ribak et al., 1978; Gabbott et al., 1986). The presence of GABAR on the granule cells was inferred by others using ³H-GABA or ³H-muscimol binding after the depletion of specific cerebellar neuronal types (Simantov et al., 1976; Chang et al., 1980; Palacios et al., 1981a). In the molecular layer, the immunoreactivity was very likely present on the surface of the Purkinje cell dendrites, but it could not be detected on the cell bodies of these cells or associated with the *pinceaux*, or baskets, that could be seen with GAD antibody (Fig. 3*C*; Oertel et al., 1981), or with anti-NFP antibody (Fig. 2*E*). The localization of the BZDR, probably on the surface of the dendrites of the Purkinje cells, could be better visualized in coronal sections that are perpendicular to the dendritic tree of the Purkinje cells (Fig. 3*B*). The Purkinje cell dendrites receive GABAergic synapses from the stellate cells. The GAD immunoreactivity (Fig. 2*C*) was also present in the granule and molecular layers and absent from the white matter. The granule cells and the Purkinje cell dendrites showed the presence of presynaptic GABAergic contacts (Fig. 3*C*). The GABA immunoreactivity is also shown in Figure 2*D*. The stellate and basket cells are GABAergic and their axons synapse the dendrites and cell bodies of the Purkinje cells, respectively. In the granule cell layer, the large Golgi II cells are GABAergic and their axons synapse the granule cells, frequently at the glomeruli (Gabbott et al., 1986).

In the deep cerebellar nuclei (Fig. 3*E*) the GABAR immunoreactivity is concentrated on boutons on the surface of neurons that receive GABAergic inputs from the Purkinje cells and from other intrinsic neurons (McLaughlin et al., 1974; Wassef et al., 1986).

Olfactory bulb

The GABAR immunoreactivity (Fig. 4*A*) was highest in both the external plexiform layer (EP) and the olfactory glomeruli (GL; Fig. 4*F*), with less reaction in the granule cell layer (GR) and the mitral cell layer (boundary between EP and GR). These results agree with those from the ligand autoradiography studies for the GABAR (Palacios et al., 1981b) and with the distribution of GAD and GABA by immunocytochemistry and biochemical methods (Fig. 4, *B*, *E*; Ribak et al., 1977). The periglomerular and tufted cells are GABAergic and form synapses in the glomeruli with the distal dendrites of the mitral cells. The granule cells from the granule cell layer are also GABAergic and send dendrites to the external plexiform layer forming GABAergic synapses with the proximal dendrites of the mitral cells. These granule cells are revealed with the anti-GABA antibody (Fig. 4*E*). The absence of immunoreactivity seen with the anti-GABA_A receptor mAb of the periglomerular area (Fig. 4*F*) shows that the receptor is present in the glomerulus, which is rich in GABA synapses, but not on the cell bodies of the periglomerular neurons. Nevertheless, periglomerular GABAergic cells show

Figure 1. Rat brain immunocytochemistry with the mAb 62-3G1. Sagittal (*A*) and coronal (*B*, *C*) sections. The fixative was PLP. The immunocytochemistry was a PAP in *A* and an ABC procedure in *B* and *C*. CO, cerebral cortex; CE, cerebellum; DLG, dorsolateral geniculate; H, hippocampus; IC, inferior colliculus; LP, lateral posterior thalamic nucleus; MG, medial geniculate nucleus; S, corpus striatum; SC, superior colliculus; SN, substantia nigra; ST, striate cortex.

Figure 2. Cerebellum immunocytochemistry with 62-3G1 diluted 1000 $\times$ (*A*, *B*), anti-GAD (*C*), anti-GABA (*D*), and anti-NFP (*E*). *B*, The mAb 62-3G1 (1 ml) was incubated with 9 μ g of the affinity-purified GABAR/BZDR complex previous to the incubation with the brain tissue. *M*, molecular layer; *P*, Purkinje cell layer; *G*, granule layer; *W*, white matter. Notice the large GABA-containing Golgi II cells in the granule layer (*C*, *D*). The brains were fixed with 4% paraformaldehyde/0.1% glutaraldehyde. Sagittal sections. Bar, 100 μ m.

anti-GAD immunoreactivity in the cell bodies (Fig. 4*G*). Some axonlike processes, like the one shown in Figure 4*D*, which was found in the boundary between the granule cell layer and the white matter, had 62-3G1 immunoreactivity.

Hippocampus

Anti-GABAR immunoreactivity was highest in the stratum oriens (SO, more in CA1 and CA2 than in CA3), followed by the

Figure 3. Cerebellum immunocytochemistry with 62-3G1 (*A, B, D, E*) and anti-GAD antiserum (*C*). All sections are sagittal except *B*, which is coronal. The deep cerebellar nucleus is shown in *E*. Notice the absence of 62-3G1 immunoreactivity on the Purkinje cell somata (*A*) and the high concentration of BZDR immunoreactivity in the cerebellar glomeruli and the lesser reactivity in the membranes of the granule cell somata (*A, D*). Notice also the GAD immunoreactivity in the *pinceaux*, or baskets, surrounding the Purkinje cells (*C*). In *A, C*, and *D*, the fixative was 4% paraformaldehyde/0.1% glutaraldehyde, and the optics were differential-interference contrast. In *B* and *C*, the fixative was PLP. Bars: 50, 20, 10 μ m (*B, C, D*, respectively). *A, C*, and *E* are shown at the same magnification.

stratum radiatum (SR) and stratum lacunosum (SL), in that order (Fig. 5, *A, B*). The stratum pyramidale had low reactivity and the structures rich in myelin fibers, such as the alveus and the fimbria, had the lowest. A few axonlike processes in the fimbria showed immunoreactivity (Fig. 6*D*). In the dentate gyrus (Fig. 5, *E, F*), the highest immunoreactivity was in the molecular

layer (M), with less in the plexiform or polymorph (P) layer. In the hilus (HL), the reactivity was even lower. The granular cell layer (G) had the least reactivity. Therefore, the quantitative distributions of the 62-3G1 immunoreactivity and ³H-muscimol binding were identical (Palacios et al., 1981b). The GABAergic cells (GABA- and GAD-positive) were distributed

Figure 4. Olfactory bulb immunocytochemistry with 62-3G1 (*A, D, F*), anti-GAD (*B, G*), anti-GABA (*E*), and the anti-nuclei monoclonal antibody 21-5E1 (*G*). *GL*, glomeruli; *EP*, external plexiform layer; *GR*, granule cell layer. Notice that very few cell nuclei are found in the glomeruli (*C*); however, they are abundant in the periglomerular area. Notice the absence of 62-3G1 reactivity in the periglomerular area (*A, F*) but the presence of GAD immunoreactivity both in the glomeruli and the periglomerular cell bodies, the latter indicated with an *arrow* (*G*). Some axonlike structures also show immunoreactivity, such as the one shown in *D* in the boundary between the granule cell layer and the white matter. In *E*, the granule cell bodies have strong reactivity with the GABA antibody. These cells extend dendrites to the external plexiform layer. The fixative was 4% paraformaldehyde/0.1% glutaraldehyde. Sagittal sections. Bars: 200, 20, 50 μ m (*C, D, F*, respectively). *A–C* are shown at the same magnification, as are *D* and *G* and *E* and *F*, respectively.

Figure 5. Hippocampus and dentate gyrus immunocytochemistry with 62-3G1 (*A, B, E, F*), anti-GABA (*C*), and anti-myelin mAb 6-4H2 (*D*). The CA3 (*A*) and CA1 (*B–D*) regions of the hippocampus, as well as the dentate gyrus (*E, F*) are shown. *AL*, alveus; *SO*, stratum oriens; *SP*, stratum pyramidale; *SR*, stratum radiatum; *SL*, stratum lacunosum; *M*, molecular layer; *G*, granule cell layer; *P*, plexiform layer; *HL*, hilus. The myelin immunocytochemistry in *D* was used to show the different layers in the hippocampus and the heavy myelination of fibers in the alveus. Notice the high immunoreactivity in the stratum oriens of the hippocampus (*A, B*) and the molecular layer in the hilar region (*F*). The PLP fixative was used in *A, E*, and *F*, and 4% paraformaldehyde/0.1% glutaraldehyde fixative in *B–D*. Sagittal sections. Bar, 100 μ m.

through all the layers of the hippocampus (Figs. 5*C, 6E*). Colchicine treatment of the rats is followed by a large increase in the number of GAD-immunoreactive cells in all layers, but especially in the horizontal cells of the stratum oriens that are on the boundary with the alveus (Ribak et al., 1978, 1986). It is in this region of the stratum oriens (mainly in CA1 and CA2) that the highest concentration of GABAR receptor immunoreactivity is also found. Individual cells having GABARs were

more easily identified in the areas where the general reactivity was the lowest, such as in the hilus (Figs. 5*F, 6B*) and the strata pyramidale (Figs. 5*A, 6A*) and granular (Figs. 5*E, 6C*), as well as the stratum radiatum (Fig. 5*A*). The GABAR immunoreactivity was also found surrounding the unstained soma of most cells of both the strata pyramidale and granular. The resolution was not good enough to determine whether this immunoreactivity was pre- or postsynaptic. The GAD immunoreactivity

Figure 6. Hippocampus and dentate gyrus immunocytochemistry with 62-3G1 (*A–D*) and anti-GAD (*E, F*). The CA3 region of the hippocampus (*A, E*), as well as the dentate gyrus (*B, C, F*), and an axonlike structure of the few that show reaction within the fimbria (*D*). *A–C* correspond to *A, F*, and *C* of Figure 5, respectively, but at higher magnification. Sagittal sections. The fixative was PLP. Bar in *D*, 50 μ m (*A–D*); 100 μ m (*E, F*).

Figure 7. Cerebral cortex immunocytochemistry with 62-3G1 (*A*), anti-GAD (*B*), anti-GABA (*C*), and the mAb 8-6A2 (*D*). Notice that the GABAR, GAD, and GABA immunoreactivities are distributed through all layers. The mAb 8-6A2 was used as a marker for neurons and for the identification of the pyramidal cells (De Blas et al., 1984). The fixative was 4% paraformaldehyde/0.1% glutaraldehyde. Sagittal sections. Bar, 100 μ m.

both inside some cells and surrounding other cells was also found in the stratum pyramidale, especially next to the stratum oriens (Fig. 6*E*). The proximal segment of the pyramidal cell axons also receives GABAergic synaptic boutons (Somogyi et

al., 1983). Basket cells that show GABAR immunoreactivity is present in the granule cell layer of the dentate gyrus, on the boundary with the plexiform layer (Figs. 5, *E*, *F*; 6*C*). These are similar to the GAD-containing basket cells (Fig. 6*F*; Ribak et

Figure 8. Substantia nigra (*A–C*) and cerebral cortex (*D*) immunocytochemistry with 62-3G1. *B*, Control in which mAb was incubated with 9 μ g of purified GABAR/BZDR complex previous to the incubation with the tissue (Fig. 2). *SN*, substantia nigra; *BP*, basis pendunculus. Notice in *C* the presence of receptor clusters on dendrites and cell bodies, and in *D* the association of the reaction product with the neuronal surface of the cortical neurons. The fixative was PLP for *A–C* and 4% paraformaldehyde/0.1% glutaraldehyde for *D*. Sagittal sections. Bar, 100 μ m (*A*, *B*); 20 μ m (*C*, *D*).

al., 1978; Seress and Ribak, 1983; Gamrani et al., 1986), which suggests a presynaptic localization of the GABA_A receptor in these cells.

Cerebral cortex

The GABAR immunoreactivity is present through all layers, especially layer I (Figs. 1, 7*A*). Intrinsic GABAergic neurons are abundant in all layers (Fig. 7, *B*, *C*). A higher-magnification photograph (Fig. 8*D*) shows that the 62-3G1 immunoreactivity has a granular aspect and concentrates on the surface of the neurons.

Substantia nigra

Here the GABAR immunoreactivity is also very abundant, especially in the pars reticulata (Fig. 8*A*), in agreement with the

results of ³H-muscimol autoradiography. The GABAR immunoreactivity is concentrated on dendrites and cell bodies. This distribution is consistent with the abundance of GAD-containing terminals synapsing dendrites and somata in this region (Ribak et al., 1976). The immunoreactivity could be blocked by purified GABAR/BZDR complexes (Fig. 8*B*).

Discussion

The distribution of the GABA_A receptor revealed by light-microscopy immunocytochemistry, using the anti-GABAR/BZDR complex mAb 62-3G1, is identical to the distribution of the high-affinity GABA_A receptor revealed by ligand autoradiography with ³H-muscimol (Palacios et al., 1980, 1981a; Penney et al., 1981; Pan et al., 1983; Schoch et al., 1985; McCabe and Wamsley, 1986). The general distributions of the GABA_A re-

ceptor shown by our mAb 62-3G1 and by another mAb to the GABAR/BZDR complex, prepared in a different laboratory (Schoch et al., 1985), were identical. Nevertheless, no high-resolution study was done with the latter antibody except in the substantia nigra, where the published results were very similar to our findings (Möhler et al., 1986). The 62-3G1 immunoreactivity colocalized better with the binding of ³H-muscimol than with the binding of ³H-benzodiazepines to the brain tissue. One of the clearest differences between the distribution of GABA_A and benzodiazepine receptors is found in the cerebellum, where the ³H-muscimol binding concentrates at the granule cell layer, while ³H-benzodiazepine binding is highest at the molecular layer (Young and Kuhar, 1979; Schoch et al., 1985; McCabe and Wamsley, 1986). The distribution of radioligand binding, as well as biochemical studies, indicates that in the membranes the low-affinity, but not the high-affinity, GABA_A receptor is functionally coupled to the BZDR. Nevertheless, the GABA_A receptor present in the affinity-purified GABAR/BZDR complex in Triton X-100 has high binding affinity for ³H-muscimol, although the functional (not physical) coupling between the receptors was lost in this detergent (Mernoff et al., 1983; Sigel et al., 1983; and the following paper, Vitorica et al., 1988). Therefore, the mAb 62-3G1 seems to recognize both the low-affinity GABA_A receptor that is functionally coupled to the BZDR and the high-affinity or functionally uncoupled form of the GABA_A receptor. We know that the mAb recognizes the coupled form of the GABA_A receptor because it immunoprecipitates all the activities of the CHAPS-solubilized receptor complex (including the benzodiazepine-, muscimol-, and TBPS-binding sites) and because GABA increases the binding of ³H-FNZ to the immunoprecipitated complex (see the following paper, Vitorica et al., 1988). We also know that the mAb recognizes the functionally uncoupled form because the distribution of the antibody binding by immunocytochemistry colocalizes better with ³H-muscimol than with ³H-benzodiazepine binding, as discussed above. These and other results suggest that (1) the 57,000 *M_r* peptide is the muscimol (GABAR agonist)-binding subunit of the complex; this interpretation is also supported by photoaffinity-labeling experiments, which show that ³H-muscimol binds to the 57,000 *M_r* peptide, while ³H-benzodiazepines bind to the 51,000 *M_r* peptide (Casalotti et al., 1986; Deng et al., 1986; Sieghart et al., 1987); (2) a large population of the GABAR is not functionally coupled to the BZDR. The functional uncoupling does not necessarily mean that the receptors are physically uncoupled. The GABAR could be physically coupled to a hidden or low-affinity form of the BZDR; and (3) the low- and high-affinity forms of the GABAR are immunologically similar, possibly the same protein in different conformational states.

In this paper we have described a high-resolution light-microscopy immunocytochemistry study with the anti-GABAR mAb 62-3G1. The GABA_A receptor concentrates in structures rich in GABA synapses, such as the cerebellar glomeruli, the external plexiform layer and the glomeruli of the olfactory bulb, the cerebral cortex, and the substantia nigra. This distribution indicates a synaptic localization of the GABA_A receptor. We do not yet know whether the immunoreactivity in these structures rich in GABA synapses corresponds to postsynaptic receptors, presynaptic autoreceptors, or both. EM immunocytochemistry, lesion experiments, and the use of neurological mutants will be valuable in providing the answer. Nevertheless, the presence of GABA_A receptors in presynaptic and possibly extrasynaptic areas is suggested by Figures 4*D* and 6*D*, where individual axonlike

processes having varicosities show GABAR immunoreactivity. Figures 5*E* and 6*C* also suggest a presynaptic localization of the GABA_A receptor, as discussed above. We feel that the GABA_A receptor is probably localized both pre- and postsynaptically.

We have found a good general correlation between the localization of GABA or GAD, on the one hand, and the localization of the GABAR on the other. Nevertheless we have also noticed some discrepancies. Differences were already noticed after radioligand autoradiography studies of the GABA_A receptor distribution (Kuhar et al., 1986). We have confirmed these findings using the anti-GABA_A receptor mAb 62-3G1. One obvious mismatch is in the region of the GABA- and GAD-rich terminals that form the *pinneaux*, or basket, that surrounds the somas of the Purkinje cells. The Purkinje cells, however, have no GABA_A receptor immunoreactivity on the surface of the somas. Another discrepancy is that the abundance of GAD-immunoreactive terminals in the stratum pyramidale of the hippocampus, in the region next to the stratum oriens, is not accompanied by an enrichment in GABA_A receptors in the same area. These discrepancies might be due to several reasons. (1) In addition to the GABA_A, there are also GABA_B receptors, which are not coupled to the Cl⁻ channel or to the BZDR (Bowery et al., 1984). These synapses would have GAD-positive terminals but they would be GABA_A receptor-negative. (2) The concentration of GABA_A receptors in these synapses might be too low to be picked up by our immunocytochemistry assays, or the conformational state of these receptors might be inaccessible or unrecognizable by the mAb 62-3G1. (3) The mismatch between presynaptic markers and the postsynaptic receptors for all neurotransmitters is a common phenomenon for which several explanations have been given (Kuhar, 1985; Kuhar et al., 1986). It could be due to the location of the neurotransmitter (GABA) and the synthesizing enzyme (GAD) in the presynaptic neuron, whereas the receptor is localized in dendrites, cell bodies, and axons of the postsynaptic neuron (Tietz et al., 1985; Trifiletti and Snyder, 1985). In addition, the GABA_A receptor is also localized in presynaptic terminals (Lo et al., 1983) having either an autoreceptor function in GABA-releasing neurons (Mitchell and Martin, 1978a, b; Snodgrass, 1978; Arbilla et al., 1979; Kuriyama et al., 1984; Tietz et al., 1985) or an extrasynaptic receptor function in both GABA- and non-GABA-releasing neurons (Levi, 1984).

Note added in proof: While this manuscript was under review, Richards et al. (1987) reported an immunocytochemical study of the distribution of the GABA_A/benzodiazepine receptor complex in the CNS using 2 of their monoclonal antibodies. This report and our study both support and complement each other.

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