Ann. Rev. Pharmacol. Toxicol. 1982. 22:245–77

DRUG INTERACTIONS AT THE GABA RECEPTOR-IONOPHORE COMPLEX

Richard W. Olsen

Division of Biomedical Sciences and Department of Biochemistry, University of California, Riverside, California 92521

Increasing evidence supports a role for the major inhibitory neurotransmitter, gamma-aminobutyric acid (GABA), in the action of many central nervous system depressant and excitatory drugs. The major type of inhibitory synaptic transmission mediated by GABA involves a rapid (milliseconds) increase in the postsynaptic membrane conductance to chloride ions (1, 2) following the interaction of GABA with its recognition site, the receptor. The rapid time-course of the GABA response indicates that the GABA receptor can be placed in a category along with the nicotinic acetylcholine receptor (and perhaps the receptors for excitatory amino acids) in which the neurotransmitter receptor is tightly coupled to an ion channel, whose opening and closing is determined by the binding of the effector ligand (neurotransmitter) at a regulatory site (the receptor) (3). Modulation of the postsynaptic GABA receptor-chloride ionophore complex appears to mediate many of the sedative, anxiolytic, anticonvulsant, and muscle relaxant actions of benzodiazepines (4–11), and also the sedative-hypnotic and anticonvulsant actions of barbiturates and related compounds (4, 5, 11–19), as well as the convulsant action of picrotoxinin and related substances (5, 11, 19). These pharmacological interactions at the organismal, tissue, and cellular level have been extended recently to the subcellular level, where in vitro binding studies indicate modulation of the GABA receptor-ionophore system by these depressant and excitatory drugs. Biochemical investigations have demonstrated the presence of two specific receptor sites, one for the benzodiazepines and one for barbiturates and picrotoxinin-like compounds,

0362-1642/82/0415-0245$02.00

that are coupled to the GABA receptor-ionophore complex (Figure 1*A*). Reciprocal in vitro chloride ion-sensitive interactions between the three drug receptors support the existence of such a complex, and may provide information on the molecular mechanism of action of these substances.

GABA-BENZODIAZEPINE RECEPTOR INTERACTIONS

GABA receptor binding in vitro using radioactive GABA was first described in 1974 (20). Under sodium ion-free assay conditions [to prevent sodium-dependent membrane uptake (19, 21)] and using frozen and thawed (22), well-washed (23) membranes [to remove endogenous GABA (24)], at 0°C (for stability), GABA binding was shown to meet many criteria of receptor identification, such as low density, appropriate affinity, and specific subcellular and tissue localization (20–25). Most importantly, sodium-independent GABA binding was shown to be inhibited by those analogues (such as muscimol, 3-aminopropane sulfonic acid, and bicuculline), and

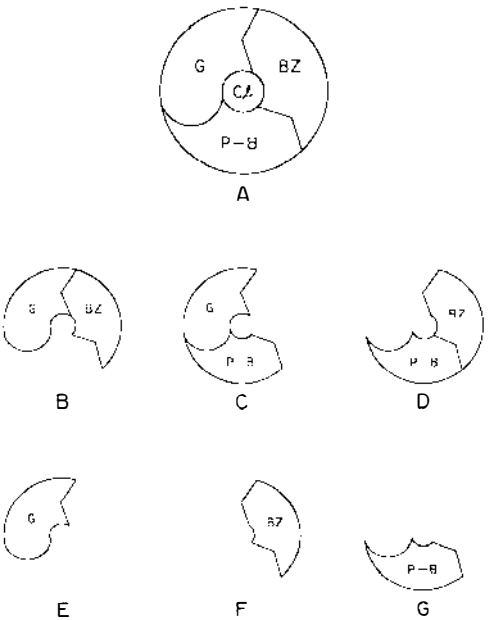

Figure 1 Schematic representation of the GABA receptor-ionophore complex and its component parts. The portion labeled G indicated the GABA receptor, BZ indicates the benzodiazepine receptor, P-B indicates the picrotoxinin-barbiturate receptor, and Cl the chloride ion channel. Each component of the three-receptor complex (*A*) can exist as individual entities (*E, F,* and *G*) or in combinations of two (*B, C,* and *D*).

only those analogues (not the GABA transport inhibitors, nipecotic acid, or 2,4-diaminobutyric acid) which are active as agonists or antagonists on GABA synapses (20, 21, 26). GABA receptor binding in brain membranes from numerous vertebrate species of various ages, and in all brain regions and subcellular fractions, was best described by two populations of different affinity, but both having receptor-like pharmacological specificity (27). These two populations gave K_D values of roughly 10–20 nM and 100–200 nM in various tissues; the density of sites (B_{max}) varied with brain region, with 0.05–0.5 pmol/mg membrane protein for the high affinity sites and 0.3–3 pmol/mg protein for the low affinity sites (27, 28). The same two populations appear to be labeled by radioactive receptor-specific GABA analogues, such as muscimol (29–31), isoguvacine (32), and piperidine-4-sulfonic acid (33), which have the advantage that they, like GABA, bind to receptor sites in the presence or absence of sodium ions, but, unlike GABA, these other ligands do not interact significantly with uptake sites in the presence of sodium. The possible roles of these two populations of GABA receptor binding sites are discussed below. For reviews of GABA receptor binding literature, see references (5, 19, 34–37).

GABA receptor binding was not significantly affected by the synaptic antagonist picrotoxinin (21, 26, 38, 39), nor by barbiturates (38, 39) or benzodiazepines (34–39). Benzodiazepine enhancement of GABA binding was observed by one group (40, 41), but only under certain prescribed conditions. Benzodiazepines did not enhance GABA binding in thoroughly washed membranes, prepared as described above (22, 23), but did so only in gently homogenized membranes subjected to little or no washing (40–42). Washing the membranes by freeze-thawing in buffer or by heating in low levels of Triton X-100 resulted in increased GABA binding but a loss of the benzodiazepine enhancement, which could be restored by readdition of the detergent washes to the washed membranes (42). The detergent washes were shown to contain a modulator protein of 15,000 mol wt which blocked high affinity GABA binding sites (42); this block could be removed specifically by benzodiazepines (40). Other laboratories so far have not been able to reproduce the isolation of a 15,000 mol wt inhibitor of GABA binding (24), nor has any other group reported any modulation of GABA binding by benzodiazepines (but see below). Further studies on the modulator protein (43, 44) suggest that it is clearly distinguishable from GABA itself and has a marked sensitivity to proteolytic degradation, which may explain the difficulties in its isolation.

The modulation of GABA binding by benzodiazepines might be expected on the basis of neurophysiological evidence linking the two (4), although the two receptors need not be directly coupled on the basis of cellular observations alone. However, this interaction is very strongly predicted on

the basis of the well-documented observation of the reciprocal interaction, namely, in vitro modulation of benzodiazepine receptor binding by GABA receptor agonists (45). Such interactions indicate that at least some GABA binding sites and some benzodiazepine binding sites are physically coupled together in the membrane, or at least can come in contact some of the time. Benzodiazepines protect GABA binding activity from inactivation by heating and protein reagents (46). This observation is consistent with a growing body of evidence that much of the benzodiazepine receptor activity in brain is directly associated with GABA receptors in the form of a large macromolecular complex, as in Figure 1*A*.

High affinity benzodiazepine binding to mammalian brain membrane fractions was described in 1977 (47, 48). [^{3}H] Diazepam was shown to label sites related to the pharmacological actions of these drugs (45, 47–52), with an apparent single affinity ($K_D \simeq 4$ nM); the number of binding sites B_{max} was consistently lower ($\simeq 1$ pmol/mg membrane protein from rat cortex) than that for GABA receptors ($\simeq 3$ pmol/mg). The binding is routinely assayed at 0°C and affinities are considerably lower at physiological temperatures (47, 53). The same sites can be labeled by the higher affinity benzodiazepine, [^{3}H] flunitrazepam, with $K_D \simeq 1$ nM (53–55); this ligand can also be used as a photoaffinity label of the benzodiazepine receptors (56). A single radioactively labeled peptide with mol wt of 51,000 was covalently labeled by this ligand upon irradiation with ultraviolet light and subsequent polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (56). High resolution gels by another group indicated 2–4 labeled peptides in the molecular weight range of 50–60,000, depending upon the brain region examined (57). Multiple peptides binding benzodiazepines would be consistent with indications of heterogeneity of binding sites for certain benzodiazepine receptor ligands. [^{3}H] Diazepam and flunitrazepam do label some medium affinity ($K_D = 20$–50 nM) sites in peripheral tissues such as kidney and liver, which have no "brain-type" binding sites (52). These "peripheral-type" sites have a pharmacological specificity differing markedly from that of the "brain-type" sites, e.g. low affinity for the potent anticonvulsant clonazepam and high affinity for the centrally inactive analogue RO5-4864; "brain-type" sites have a high affinity for clonazepam and low affinity for RO5-4864 (52, 58). The "peripheral-type" sites appear to be the only sites detectable in cloned cell lines of glial or neuronal origin [(58); R. W. Olsen, unpublished observations; M. Karobath, personal communication]; primary cultures of nerve (59, 60) but not glial cells (59, 61) contain "brain-type" binding sites. "Peripheral-type" sites also exist in brain and may be detectable at high concentrations of ligand, indicating a second binding site population $K_D = 20$–50 nM (62), in addition to the 5 nM K_D site mentioned above; this second site, however, does not show the

ligand specificity correlating with central pharmacological activity of the benzodiazepines, so it is not truly a second example of "brain-type" benzodiazepine receptors (63).

The "brain-type" high affinity binding sites seem to bind most active benzodiazepines with an apparent single affinity, i.e. no curvilinear Scatchard plots or low Hill numbers (64, 65). However, the displacement of [^{3}H]diazepam or [^{3}H] flunitrazepam binding by certain drugs shows a shallow concentration dependence suggestive of heterogeneity of binding site affinity, i.e. possible multiple receptor sites, although an allosteric conversion of the benzodiazepine receptor into a negatively cooperative system is an alternate explanation. The anxiolytic triazolopyridazine agents such as CL 218,872 were the first examples of substances showing shallow displacement curves, the slopes of which differ with brain region (64). These authors suggested that the triazolopyridazines bind to the same sites as benzodiazepines, but with differing affinities for two populations of sites which are not distinguishable using benzodiazepines as ligands. Computer analysis of displacement data indicated two distinct affinities for CL 218,872 (K_D = 70 nM and 3 μM), with the fraction of high affinity sites varying from about 95% in cerebellum to 50% in cortex (66). Binding of radioactive, CL 218, 872 confirmed this interpretation, labeling two populations of sites whose ratios varied with brain region in a manner consistent with the potency of this drug to displace [^{3}H] flunitrazepam binding (67). Histochemical autoradiography on brain slices using [^{3}H] flunitrazepam (68) further demonstrated that there are two populations of benzodiazepine receptors having a different brain regional distribution: those that were displaceable by low concentrations of CL 218,872 (site 1) and those not displaceable (site 2). A differential ontogeny for these two types (69) also demonstrates that they represent two distinct receptors.

A very similar situation exists with respect to another class of benzodiazepine receptor ligands, the β-carbolines, which are putative endogenous ligands for the receptors (65). These compounds also show shallow displacement curves against [^{3}H] diazepam or flunitrazepam, suggestive of heterogeneity. The degree of heterogeneity again depends on the brain region e.g. shallower curves in hippocampus than cerebellum (70). Studies with [^{3}H] propyl-β-carboline-3-carboxylate show a labeling of only the high affinity subpopulation, which has a B_{max} almost equal to that for sites labeled by [^{3}H] flun
cantly lower B_{max} than [^{3}H] flunitrazepam in other areas like forebrain (71). First approximations suggest that the two affinity subpopulations for triazolopyridazines and β-carbolines may correspond, i.e. high affinity CL 218,872 sites may be equal to high affinity sites for β-carbolines. Because

of the relative homogeneity of both β-carboline binding sites (71) and of the 41,000 mol wt affinity-labeled peptide in cerebellum (57), this peptide might correspond to the high-affinity β-carboline binding receptor, with the other photoaffinity-labeled peptides corresponding to lower affinity sites (71).

Heterogeneity of benzodiazepine binding sites was also seen in the reaction of an affinity label kenazepine, which irreversibly inactivated only a fraction of the total binding sites (72). Further evidence for heterogeneity comes from the nonlinear kinetics of thermal inactivation of benzodiazepine binding activity (64), and the even more pronounced heterogeneity in the protection against such loss of activity by certain ions and by GABA receptor ligands (73).

This protection by GABA against the heat inactivation of benzodiazepine receptor binding (64) was the first evidence for a physical coupling between GABA and benzodiazepine receptors. The pronounced biphasic heat inactivation kinetics even in the presence of GABA suggested that all benzodiazepine binding sites might not be equally well coupled to GABA receptors (73). At the same time, Gallager et al (74) observed that drugs which elevated GABA levels in vivo caused an increased benzodiazepine binding subsequently assayed in vitro. This effect was apparently due to increased levels of GABA in the brain extracts assayed, and led to the discovery that GABA enhanced benzodiazepine binding in vitro (75). The enhancement was due to a reversible increase in affinity of about twofold, with no change in the number of binding sites (53, 75–80); the K_D decrease reportedly was due to an increased binding association rate (75) or to a decreased dissociation rate (53). The enhancement could be detected by histochemical autoradiography in brain slices (81) and in biochemical assays of membranes subjected to thorough washing (43, 73, 82, 83) or even in detergent-solubilized benzodiazepine binding proteins (84), indicating that some GABA receptors are tightly associated with some benzodiazepine receptors.

Bicuculline reversed the GABA enhancement of benzodiazepine binding (75), and also lowered the base line binding, possibly by inhibiting the effect of endogenous GABA, but allosteric inhibition in the absence of GABA is a possibility. Although several well-known GABA agonists like muscimol mimicked GABA in enhancing benzodiazepine binding, others such as THIP (4,5,6,7-tetrahydroisoxazolo [5,4-*c*]pyridin-3-ol) and piperidine-4-sulfonic acid did not (85, 86), suggesting at first that the GABA "receptor sites" involved in this interaction were different from the GABA receptor binding sites discussed above. Subsequent studies showed that all GABA analogues active on the latter GABA binding sites did interact with benzodiazepine binding; some compounds, however, did not enhance but

rather inhibited the effect of those GABA analogues which did enhance benzodiazepine binding (82, 87). Others gave a lower maximal enhancement than GABA and when present in excess could inhibit the action of GABA down to their own level of maximal effect, much in the manner of "partial agonists."

The relative potencies of these compounds to have some effect, i.e. enhancement or block of enhancement on benzodiazepine binding, correlated very well with their potencies to inhibit GABA binding (82), thus there is no need to evoke any new class of GABA receptors. However, the concentration dependence curves were all shifted tenfold to the right for the former effect (75, 82): the concentration of GABA required for 50% of the maximal enhancement was 0.4–3 μM, as opposed to 0.1–0.2 μM for the *lower* affinity subpopulation of GABA binding sites measured under similar conditions (27, 36, 82). The absolute potencies in these assays depend to a certain extent on the method of membrane preparation and assay (21, 23, 37, 43, 78, 82), and the use of more physiological assay conditions such as chloride-ion containing media and 37°C seems to bring the concentration dependence of the two effects closer together and to augment the ability of "inactive" GABA analogues to enhance benzodiazepine binding (88). A higher potency for GABA or muscimol enhancement of benzodiazepine binding more in correspondence with binding affinities has been reported for special cases, which appear to be the exception rather than the rule. Examples include assays done in the presence of certain allosteric activating drugs (89, 90), in human brain but not other species (91), in detergent-washed membranes (43), and in certain solubilized preparations (92). Some groups have proposed that thorough washing improves the potency for GABA enhancement of benzodiazepine binding in parallel to the improved potency of GABA binding (43), but this is not universally observed [(36, 78, 82); R. W. Olsen, unpublished]. It has been pointed out that those GABA agonists which best enhance benzodiazepine binding show more improvement in binding affinity after thorough membrane washing than those which do not enhance benzodiazepine binding; since washing appears to affect primarily high affinity GABA sites, these were deduced to be the population which is coupled to benzodiazepine receptors (93). However, examination of a large number of GABA analogues does not support this interpretation (21, 36, 82), and furthermore, several other lines of evidence suggest that those GABA sites associated with benzodiazepine receptors are a rather low affinity population. In the great majority of cases studied using identical membranes and assay conditions, the apparent affinity for direct GABA binding was greater than for the indirect effects on benzodiazepine binding, and this discrepancy still needs explanation.

It may be pertinent to mention that whenever dose-dependence for

GABA analogues to produce a physiological response in intact tissue has been measured (11, 14, 94), even higher concentrations (10–100 μM) of GABA seem required. Nevertheless the potencies of a series of GABA analogues to open chloride channels on cultured mouse spinal cord neurons, as indicated by variable life-time channels during fl
correlated highly with potencies on GABA binding studies at 0°C on vigorously homogenized and washed mammalian brain membranes, although giving at least 100-fold difference in absolute concentration dependence. Nevertheless, in this case, unlike the reciprocal GABA and benzodiazepine studies, quite different assay conditions were employed in the binding studies and the electrophysiological experiments on intact cells. One might conclude that the tissue homogenization and nonphysiological medium and temperature caused a shift of the receptor conformation to an "artificially" higher affinity state, remarkably parallel in affinity for a series of ligands.

Such a conformational shift probably does not explain the discrepancy in GABA affinities for binding and enhancing of benzodiazepine binding. Although the vigorous homogenization and membrane washing procedures may indeed disturb the native membrane protein structure and environment, both activities are observed in these membranes and both might be expected to be affected in a similar manner. A reasonable theory is that the GABA binding sites which are coupled to benzodiazepine binding sites represent a population of low affinity sites ($K_D \simeq 1$ μM) which are normally not readily detected in direct binding. These hypothetical sites have a specificity remarkably similar to the detected sites and so might represent another conformational state of the detected sites. These difficult-to-detect sites might be modulated by benzodiazepines, but apparently would not be enhanced sufficiently to make them more detectable. It is also possible that some nonequilibrium situation exists, such that the state of the GABA receptor when it interacts with the benzodiazepine receptor is not the same state measured at equilibrium; no experimental evidence supporting this has been reported, however. Whatever the explanation, some GABA binding sites clearly are allosterically coupled to benzodiazepine sites.

Another interesting aspect of the interaction involves the chloride ion channel associated with GABA receptors. GABA binding has been reported to be perturbed by certain anions, primarily involving a large (10–100-fold) increase in affinity for the GABA antagonist bicuculline (95) but also a small decrease in GABA agonist binding affinity (96). The effective anions included SCN^-, I^-, and NO_3^-.

Certain anions also enhanced benzodiazepine base line binding (77, 97, 98), as well as improving the enhancement by certain GABA analogues (88). GABA enhancement of [^{3}H] flunitrazepam binding was very much

augmented by chloride ions when assayed at 37°C, although the effect was small at 0°C (99). Anions which enhanced benzodiazepine binding included Cl^-, Br^-, I^-, NO_2^- SCN^-, ClO_4^-, but not F^-, CH_3COO^-, sulfate, formate, succinate, propionate, or acetate (98). The specificity of the anion enhancement was proposed (98) to represent coupling of the benzodiazepine receptors to a chloride ion channel. This would presumably be the GABA chloride channel since GABA also modulates benzodiazepine binding, and the effective anions were those shown by Eccles and colleagues (100, 101) to permeate channels involved in postsynaptic inhibitory potentials in spinal cord and hippocampus, possibly GABA-regulated. This interpretation was questioned by others (102) who indicated that the variable enhancement by different anions could not be correlated to inhibitory synaptic channel permeabilities, and furthermore that defined GABA-mediated inhibitory currents in cortex (the tissue used in binding studies) showed a much broader anion specificity (103). Whereas the definitive answer will require more quantitative information, the qualitative anion specificity of the benzodiazepine binding appears relevant to the GABA chloride channel in light of the corresponding anion effects on GABA receptors and barbiturate receptor interactions with the benzodiazepine-GABA system, as described below.

The anion effect on the GABA receptors is a shift toward higher affinity for bicuculline (95, 96). Since bicuculline has a lower relative affinity for high affinity GABA sites (104), the anion shift for GABA agonists would be toward lower affinity (which, could be argued, might be the more functionally relevant). Thiocyanate at 50 mM was shown to likewise enhance the affinity of bicuculline for blocking GABA enhancement of benzodiazepine binding (from 5 to 0.05 μM), without an effect on the GABA potency ($\sim$ 1 μM). Since GABA high affinity ($K_D \simeq 10$ nM) sites were decreased under these conditions, the high affinity sites could not be mediating the interactions of GABA with benzodiazepine binding sites (96).

A similar conclusion has been obtained from anatomical studies. Benzodiazepine binding showed a similar but not identical subcellular (54, 105) and brain regional (48, 49, 105, 106) distribution to GABA binding [a mixture of high ($K_D \simeq$ 10–20 nM) and low ($K_D \simeq$ 100–200 nM) affinity sites]. Both (brain-type) benzodiazepine (56, 59–61, 107–109) and GABA (110–112) binding appear to be specific for neurons. Lesion and localization studies indicated parallel distribution of the two receptor types in some areas, e.g. retina (113–120) and striatum (121–123). Lesions of the striatonigral GABAergic pathways led to an increase in GABA binding B_{max} in the substantia nigra (124, 125); benzodiazepine binding was not increased but showed a decrease in affinity (126) and increased sensitivity to GABA enhancement (127), perhaps due to decreased endogenous GABA. A larger

discrepancy occurred in the cerebellum between GABA and benzodiazepine binding distribution.

Lesions of the cerebellum (109, 128, 129) and mutants depleted of certain types of cerebellar cells (109, 130–132) indicate that GABA binding sites are numerically most dense on granule cells (innervated by inhibitory Golgi II type cells), whereas benzodiazepine binding sites are not correspondingly localized but are proportionally more dense on Purkinje cells. This discrepancy appears to be due to a high incidence of GABA binding sites of high affinity which are not associated with benzodiazepine receptors. A quantitative study of histochemical autoradiography of benzodiazepine binding and muscimol binding in brain slices from various regions revealed no significant correlation in distribution of these sites (133); the cerebellum was particularly different, with GABA binding concentrated in the granule layer and benzodiazepine binding in the molecular layer (81, 107, 133, 134). Nevertheless, at the same time, benzodiazepine binding, wherever it was observed throughout the brain, was always enhanced by GABA, and to a quantitatively similar level (133), indicating that most benzodiazepine binding sites are associated with some GABA receptors, although apparently not the high affinity GABA/muscimol binding sites which dominate the binding/autoradiography studies on those sites.

Further support for the conclusion that some GABA binding sites are not associated with benzodiazepine receptors comes from ontogeny studies on the two receptor types. In the rat, a greater fraction of adult levels are present at birth for benzodiazepine receptors than for GABA receptors, followed by a parallel increase in both (25, 135–139). However, the GABA enhancement of benzodiazepine binding develops in very close correspondence with the base line benzodiazepine binding itself rather than with the overall GABA binding (137–139), indicating again that benzodiazepine binding and overall GABA binding do not correspond, and that GABA enhancement is due either to a low affinity or undetectable subpopulation of GABA receptors.

Conversely, there is less evidence for benzodiazepine binding sites which are not associated with GABA receptors. Besides the uniform GABA enhancement of benzodiazepine binding with region (106, 133), GABA also enhanced the binding of [^{3}H] flunitrazepam to all of the peptides photoaffinity labeled (57). Using another marker of GABA synapses, the synthetic enzyme glutamic acid decarboxylase (GAD), immunocytochemical localization of this enzyme at the electron microscope level, coupled with autoradiography using the photoaffinity label [^{3}H] flunitrazepam, revealed that at least one third of the benzodiazepine receptors labeled were associated with GAD-positive synapses (140). It could not be conclusively determined whether the other two thirds were similarly situated.

Furthermore, despite the apparent heterogeneity in binding of some benzodiazepine receptor ligands such as CL 218,872 (66–69), both of the two different affinity subpopulations for this ligand are enhanced by GABA as demonstrated both by autoradiography (68) and membrane binding (66). On the other hand, the binding of β-carbolines to benzodiazepine receptors is little enhanced by GABA (71, 99), suggesting a possible receptor type uncoupled to GABA receptors. A more likely explanation for the low or absent GABA enhancement of β-carboline binding comes from the evidence that β-carbolines have the opposite, or antagonistic, pharmacological activity as the benzodiazepines (99), and thus might respond differently to allosteric ligands acting at sites on functionally associated effector proteins.

Soluble receptor studies also indicate the existence of a GABA-benzodiazepine receptor complex. Early reports of GABA receptor solubilization (31, 141, 142) and benzodiazepine receptor solubilization (142–144) did not mention concomitant solubilization of the other receptor. We (31) observed an optimal solubilization of GABA/muscimol binding with 2% deoxycholate yielding a species of apparent molecular weight on Sepharose 6B columns of 900,000 (compared to globular standards). This material contained no detectable benzodiazepine binding activity (34), had an apparent single K_D for muscimol and GABA binding of roughly 50 nM. The yield of less than 100% solubilization (about 30%) left open the possibility that a subpopulation, perhaps the low affinity sites, had been selectively solubilized (31, 34). The detergent-extracted membranes retained about 30% of the original binding, with the same two apparent binding affinities still present (34). It is also possible that the two apparent binding affinities in membranes reflect a single receptor protein with different environmental restraints which are no longer a factor in the solubilized state.

Solubilized benzodiazepine receptor was reported to sediment at 12*s* on sucrose gradients (143) and to chromatograph with an apparent molecular weight of 200–300,000 on sizing columns (144), and to show no enhancement by GABA. GABA and benzodiazepine binding activities were reported to show differential sensitivity to (145), and solubilization by (44, 146), various detergent treatments, suggesting they either reside on separate macromolecules or can become separated. One report of column separation of the two binding activities has appeared (147). Nevertheless, since both types of binding activity appear to consist of at least two subpopulations, it is possible that some populations are coupled even if all are not.

Soluble benzodiazepine binding activity which was enhanced by GABA ($\sim 1\ \mu M$) has been obtained by several workers (84, 146, 148, 149). Chloride enhancement of soluble benzodiazepine binding has also been reported (148, 150). Extracts showing GABA enhancement invariably contained GABA receptor binding activity as well (92, 146, 150–152). The majority

of the GABA and benzodiazepine binding activities were observed to comigrate on sucrose gradients (92, 146, 151, 152) (with s values of 9–12, depending on the detergent), on sizing columns (92, 146, 150) (with Stokes radius equal to globular standards of 700,000 ± 200,000), on ion-exchange columns (92, 150), on lectin-agarose columns (150), and on a benzodiazepine affinity column (150). These samples contained roughly equivalent amounts (within a factor of 2) of the two types of binding sites (44, 92, 146, 150, 152). GABA binding to soluble receptors was generally reported to give K_D values in the range of 30–60 nM (92, 146, 150); enhancement of benzodiazepine binding required higher concentrations ($\simeq$ 500–1000 nM) of GABA (84, 150), although one report of enhancement at 50% of maximal by concentrations as low as 73 nM has appeared (92). One preparation treated with the photoaffinity label [^{3}H] flunitrazepam
tive peptides of mol wt 55,000 and 62,000 on polyacrylamide gel electrophoresis in sodium dodecyl sulfate (150).

Using sucrose gradient centrifugation in H_2O and D_2O, we measured a 12.5s sedimentation coefficient for the complex in 0.5% Triton X-100 in both media, indicating a $\bar{v}$ of 0.73. These parameters and a Stokes radius of 6.9 nm measured on gel filtration columns allowed calculation of a mol wt of 355,000 for the "native" protein under these conditions (146). This estimate applies to the binding species in solution, which could contain bound detergents and lipids, although the identical sedimentation behavior in H_2O and D_2O suggests that the protein contributes most of this mass. In the membrane-bound state, GABA and benzodiazepine binding activities were found to have an identical functional mass of about 215,000 by the method of irradiation inactivation target analysis (152), consistent with observations that both activities reside, at least in part, on the same macromolecule.

Benzodiazepine and GABA receptor binding can also be modulated in vitro by several other agents which appear to act via at least one other associated drug receptor, the barbiturate/picrotoxinin receptors.

BARBITURATE/PICROTOXININ-BENZODIAZEPINE RECEPTOR INTERACTIONS

Barbiturate enhancement of GABA postsynaptic responses (4) for many years could not be correlated with any biochemical effects on in vitro GABA receptor binding (34, 38), but recently barbiturates have been found to show pharmacologically specific modulation of both benzodiazepine and GABA receptor binding.

Barbiturates, including those with convulsant, sedative-hypnotic, and anticonvulsant activity, competitively inhibited the binding of [^{3}H] α-dihydropicrotoxinin (DHP) to brain membrane sites (153). [^{3}H] DHP labels

sites which are related to the convulsant action of picrotoxin, a small molecular weight plant toxin which is a universal blocker of postsynaptic GABA chloride conductance responses, but which does not inhibit GABA receptor binding (38, 39). [^{3}H] DHP binding sites were inhibited by biologically active picrotoxinin analogues (154) and by bicyclic cage convulsant compounds (155) which have also been shown to block GABA postsynaptic responses. [^{3}H] DHP showed a K_D of 1–2 μM for binding to crayfish muscle (156) and mammalian brain (154), but not for nonneuronal tissues; the number of sites in the richest tissue of rat brain, cerebral cortex, was 5–10 pmol/mg membrane protein (154). [^{3}H] DHP binding sites appear to be distinct from GABA and benzodiazepine receptor sites by various criteria, while showing a similar ontogenetic development, brain regional and subcellular distribution (157).

[^{3}H] DHP binding was inhibited by crude aqueous brain extracts (158), suggesting a possible endogenous ligand for this exogenous drug receptor. The inhibitor has not yet been identified, but [^{3}H] DHP was found to be competitively inhibited by natural substances such as certain purines (adenine, hypoxanthine, inosine, and uric acid), pyrimidines (cytosine), and the purine metabolite allantoin, with potencies of about 0.1 mM (158). Interestingly, inhibition of benzodiazepine binding by brain extracts has been shown to be due, at least partly, to inosine and hypoxanthine, at about 1 mM (159–162), and perhaps nicotinamide, at 3 mM (162). Similar purines have been reported to noncompetitively inhibit GABA binding at 1 mM (163), perhaps due to an allosteric effect via the benzodiazepine or DHP sites.

The similar effects of purines on benzodiazepine and DHP binding might be due to overlap in specificity or to allosteric action at one of the sites. Picrotoxin does not block [^{3}H] diazepam binding (47, 105), but diazepam (1 μM) inhibits [^{3}H] binding. [^{3}H] DHP binding was inhibited by a series of benzodiazepines with both chemical- and stereo-specificity (105); the activity did *not* correlate, however, with potencies at the high affinity benzodiazepine binding sites or pharmacological actions of these drugs.

A convulsant benzodiazepine, RO5-3663, which blocks GABA synapses (164) rather than potentiating them like diazepam (4), at 0.1 μM was the most potent benzodiazepine inhibitor of [^{3}H] DHP binding (105); this compound inhibited [^{3}H] diazepam binding only at concentrations over 10 μM (105), and probably exerts its actions via the picrotoxinin receptor.

Among the barbiturates, the convulsant analogues also most potently displaced [^{3}H] DHP binding, at submicromolar concentrations (153). The relative and absolute potencies of hypnotic barbiturates (1–100 μM) and anticonvulsant barbiturates (1–1000 μM) correlated roughly (153) with pharmacological activity for central nervous system depression and en-

hancement of GABAergic postsynaptic responses (12, 14). Definitive correlations were made difficult by the scarcity of dose-response data for barbiturate activity and the technical problems inherent in [^{3}H] DHP binding assays due to the relatively low affinity of this ligand (no higher affinity radiolabeled ligand for the same sites has yet been described).

[^{3}H] DHP binding was also inhibited by some other interesting depressant drugs, including diphenylhydantoin [100 μM, (154)], chlormethiazole [500 μM, (165)], and etazolate = SQ 20009 [5 μM, (83)]. The latter compound, an example of anxiolytic pyrazolopyridine drugs, provided an important link between barbiturate/picrotoxinin binding sites and the benzodiazepine-GABA system. Etazolate (166) and related anxiolytics cartazolate (167) and tracazolate (168) did not inhibit [^{3}H] diazepam binding, but enhanced it. These drugs did not act at GABA receptors, and the enhancement of benzodiazepine binding was additive, or even synergistic, with GABA enhancement (89). The enhancement of benzodiazepine binding by etazolate was potentiated by chloride ions and inhibited by picrotoxin (169). Etazolate action is mediated via a picrotoxinin site, since the block of etazolate enhancement by picrotoxinin occurred at concentrations (micromolar) similar to those displacing [^{3}H] DHP binding, and etazolate inhibited [^{3}H] DHP binding at concentrations (micromolar) similar to those enhancing benzodiazepine binding (83).

Like picrotoxinin, other excitatory agents which inhibit [^{3}H] DHP binding, such as isopropyl bicyclophosphate (IPTBO), tetramethylene disulfotetramine (TETS), pentylenetetrazole, and the benzodiazepine R05-3663 also inhibited etazolate enhancement of benzodiazepine binding (83, 170). Similarly, like etazolate, other depressant drugs which inhibit [^{3}H] DHP binding, such as the barbiturates, enhanced benzodiazepine binding (83, 171).

Barbiturates such as pentobarbital caused a reversible, concentration-dependent ($EC_{50} \simeq 100$ μM) increase in the binding affinity for [^{3}H] diazepam of over twofold, with no effect on the B_{max} (171–173). This effect occurred in all brain regions with small variations in maximal enhancement (F. Leeb-Lundberg and R. W. Olsen, submitted). Barbiturate action was additive with the enhancement by just-maximal levels of GABA, but not additive with the enhancement by etazolate, indicating action at a mutually exclusive site (83). Whereas the GABA enhancement was blocked by the nonenhancing analogues imidazole-acetic acid and THIP, these agents do not block etazolate or pentobarbital enhancement (83), indicating that the latter agents, as expected, did not act at GABA receptors. Etazolate and pentobarbital enhancement were, however, reversed by the GABA antagonist bicuculline (83, 172, 173), apparently by an allosteric interaction involving coupling to GABA receptors. Both were also competitively blocked

by micromolar picrotoxinin, consistent with action at a picrotoxinin-sensitive site (83, 170–173). Pentobarbital enhancement of benzodiazepine binding was preserved, although diminished, in detergent-solubilized samples (146, 174).

Pentobarbital caused a dose-dependent effect on the kinetics of [^{3}H] diazepam binding, decreasing both the association and dissociation rates, with the latter predominating (175), consistent with the decrease in equilibrium dissociation constant K_D. The enhancement effect was strictly dependent on the presence of chloride or certain other anions, with both the concentration dependence and maximal effect of barbiturates affected; half-maximal activation by Cl^- occurred at about 30 mM, with concentrations over 100 mM required for the maximal effect (171). Other anions which supported the barbiturate-benzodiazepine interactions, some of which were more potent than Cl^-, were I^-, Br^-, ClO_4^-, NO_3^-, SCN^-, and $HCOO^-$, but not N_3^-, $SO_4^=$, $SO_3^=$, F^-, bicarbonate, phosphate, acetate, or propionate (171). A similar anion specificity (with the exception of formate) was observed for the dependence of etazolate enhancement of benzodiazepine binding (170). As mentioned above, this is the exact anion specificity observed for the permeability of chloride channels mediating inhibitory postsynaptic potentials in various parts of the nervous system (100, 101) [with the one exception of azide, which may have had indirect actions in the cellular assays as suggested by the authors, (100)]. Therefore, some relationship of these in vitro barbiturate effects to the enhancement by barbiturates of those same inhibitory Cl^- channels in brain (13) seems evident.

The sites at which barbiturates exert these chloride-dependent, picrotoxinin-sensitive effects on benzodiazepine receptors show a chemically specific and stereospecific profile which correlates with the pharmacological activity of barbiturates. Benzodiazepine binding was enhanced by a series of depressant barbiturates, with a potency rank of 5-ethyl,5-(1,3-dimethylbutyl) barbiturate (DMBB) > secobarbital > (–)pentobarbital > (–)mephobarbital > (+)hexobarbital > (+)pentobarbital > amobarbital > (–) N^1-methyl, 5-phenyl, 5-propyl barbiturate (MPPB) > 5-ethyl, 5-cyclohexylidene-ethyl barbiturate (CHEB) > (–) hexobarbital; inactive compounds included phenobarbital, metharbital, barbital, (+)MPPB, and (+)mephobarbital (171, 175, 176). Comparison of these potencies (Table 1) with action at reversing antagonists of GABA responses on mammalian sympathetic ganglia (14) or for inducing sleep in intact animals (177) showed a high correlation (171).

Barbiturates did not all enhance to the same maximal extent. A series of analogues of pentobarbital, such as DMBB, secobarbital, and amobarbital, enhanced to the same level as pentobarbital (about 120%), with different EC_{50} values. These compounds are all potent hypnotic agents (175). Some

Table 1 Drugs active at picrotoxinin-barbiturate receptors in rat brain

Drug[a]	[^{3}H] DHP binding IC_{50} (μM)[b]	[^{3}H] Diazepam binding[c] EC_{50} (μM): Enhancement	[^{3}H] Diazepam binding[c] IC_{50} (μM): Block enhancement
Convulsants			
Picrotoxinin	0.4	n.a.	2
TETS	4	n.a.	6
IPTBO	8	n.a.	20
RO5-3663	0.1	n.a.	2
Pentylenetetrazole	30	n.a.	250
Depressants			
"Agonists"[c]			
(±) DMBB	0.1	80 (125%)	n.a.
(±) Secobarbital	5	100	n.a.
(–) Pentobarbital	22	120	n.a.
(±) Pentobarbital	50	130	n.a.
(+) Pentobarbital	82	300	n.a.
Amobarbital	n.d.	300	n.a.
CHEB	0.7	500-1000	n.a.
"Partial Agonists"[c]			
(–) Mephobarbital	6	50 (65%)	n.a.
(+) Hexobarbital	2.5	150 (80%)	n.a.
(–) MPPB	1.1	100 (35%)	n.a.
(–) Hexobarbital	3.5	> 1 mM	n.a.
Barbital	50	inactive	inactive
"Antagonists"[c]			
Phenobarbital	400	> 1 mM	500
Metharbital	10	inactive	500
Chlormethiazole	500	inactive	400
Diphenylhydantoin	140	inactive	250
(+) MPPB	0.9	inactive	100
(+) Mephobarbital	1.5	inactive	200
Pyrazolopyridines			
Cartazolate	0.5	0.4	n.a.
Etazolate	8	0.9 (65%)	n.a.

[a] Full names of abbreviated drugs are given in the text.

[b] All data are from Refs. (153, 171, 175, 176). n.d. refers to "not determined"; n.a. means "not applicable"; inactive means the compound had no effect at 1 mM or at its solubility maximum.

[c] Agonists, partial agonists, and antagonists refer strictly to interactions in vitro with benzodiazepine receptor binding. Values in parentheses refer to the maximal enhancement over control binding in absence of drug.

of these agents contain asymmetric carbons in their side-chains, and the (+) isomers are not only less potent depressants than the (–) isomers but may also have excitatory actions on nerves (178). (+)DMBB and CHEB resemble (+)pentobarbital in that, in addition to excitation, they show depressant action, which, like (–)pentobarbital, probably involves modulation of

GABA chloride ion channels (178). We speculate that the enhancement of benzodiazepine binding is related to this latter action and the excitatory effects of these drugs may involve other sites of action.

Another series of barbiturates having N^1-methyl ring structures, and therefore an asymmetric carbon at C^5, such as mephobarbital, hexobarbital, and MPPB, show distinct stereospecific interactions with benzodiazepine binding which are also different from that of the pentobarbital series. In each case, the active hypnotic isomer of the pair, (+) hexobarbital, (–)mephobarbital, and (–)MPPB, enhances benzodiazepine binding (Table 1). However, the enhancement reaches a plateau at levels significantly lower (35–80%) than for pentobarbital (175, 176). Including an excess of one of these drugs concurrently with pentobarbital lowers the enhancement of benzodiazepine binding down to the level of the lower maximal enhancement, in a manner resembling the action of "partial agonists" (as observed above for GABA enhancement).

The other isomers had two different effects. (–)Hexobarbital had no effect on either base line or pentobarbital-enhanced benzodiazepine binding. Barbital shared this lack of activity, which appears to correlate with low pharmacological activity. (+)MPPB, however, a pure excitatory analogue, and (+)mephobarbital (pharmacology unknown) reversed with a shallow displacement curve the enhancement by pentobarbital of benzodiazepine binding (175, 176), an action which, like picrotoxinin, may be related to convulsant activity.

Some other substances which inhibited [^{3}H] DHP binding but did not enhance benzodiazepine binding were also able to reverse the pentobarbital enhancement, indicating an interaction at the same barbiturate receptor site (165). Phenobarbital, for example, caused a competitive ($K_I = 200\ \mu M$) reversal of pentobarbital enhancement. With no affect on B_{max}, phenobarbital shifted the benzodiazepine binding K_D which was lowered by pentobarbital back towards the unenhanced control level (165). Phenobarbital, like pentobarbital, gave a concentration-dependent modulation of the kinetics of benzodiazepine binding, although in this case the decrease in association and dissociation rates were approximately equal, consistent with the small effect on equilibrium binding (175). It is interesting that this commonly employed anticonvulsant drug should also perturb benzodiazepine binding via the barbiturate receptor site and in a qualitatively different manner from the barbiturates commonly used as hypnotics. Some physiological studies also suggest qualitatively different actions for barbiturates with primarily anticonvulsant or hypnotic clinical uses (11, 12, 17). It is not yet known whether the in vitro reversal of pentobarbital effects, which resembles the effect of convulsants like picrotoxinin, can be related to anticonvulsant activity. Interestingly, several other nonbarbiturate depressants used as anticonvulsants have actions like phenobarbital on reversing pentobarbital

or etazolate enhancement of benzodiazepine binding. These include chlormethiazole (165), metharbital, trimethadione, ethosuximide, carbamazepine, and diphenylhydantoin, but not valproic acid, at concentrations of 100–1000 μM (176). The last three active agents also had significant effects on base line benzodiazepine binding at these admittedly high concentrations. The possible pharmacological relevance of these effects will require further study.

The data in Table 1 point out that the concentration dependence for barbiturates to affect benzodiazepine binding in a pharmacologically relevant manner does not agree very well with potencies to inhibit [^{3}H] DHP binding. Considering that picrotoxinin and related convulsants competitively inhibit the barbiturate effects at concentrations similar to their binding affinities (83, 171–173), it appears that [^{3}H] DHP labels these sites but apparently some other sites as well. The subpopulation which is coupled to benzodiazepine receptors has a relatively low affinity for barbiturates including DMBB (Table 1); other [^{3}H] DHP binding sites with relatively high affinity for some convulsants may not be coupled to benzodiazepine receptors and perhaps not even to GABA receptors.

Several other agents have been found to enhance benzodiazepine binding at so far unidentified sites. The antihelminthic agent, avermectin, irreversibly enhanced benzodiazepine binding (178a) by an increase in both affinity and in B_{max}. This agent has several pharmacological actions (none of which have been reported in mammals) including activation of chloride channels in invertebrate muscle (179). The enhancement by avermectin was not sensitive to picrotoxinin (180), slightly sensitive (180) or insensitive to bicuculline (181), and not mediated by GABA receptor sites (181, 182), suggesting that an additional receptor site for this drug is required. An increase in B_{max} for benzodiazepine binding in vitro was also seen in the presence of an unusual hydrophobic purine analogue, EMD 28422 (183), which, like other purines, might act via the barbiturate sites, or perhaps via the avermectin sites.

Ethanol has been reported by one group to enhance benzodiazepine binding at 20 mM (184), probably via the picrotoxinin-barbiturate receptor sites. This in vitro effect, although weak, may prove interesting. The ethanol- and barbiturate-sensitive [^{3}H] DHP binding activity could be solubilized in Lubrol (185) and separated from the benzodiazepine receptors by column chromatography (147).

Diphenylhydantoin was reported to inhibit benzodiazepine binding with an affinity (0.9 μM) enhanced by GABA (186). We have found this drug to be only a much weaker ($IC_{50} \simeq 300$ μM) inhibitor of base line benzodiazepine binding, while inhibiting pentobarbital enhancement and [^{3}H] DHP binding at 100–200 μM (Table 1). On the other hand, diphenylhydan-

toin in vivo led to an increase ($\simeq$ 25%) in the *number* of binding sites for benzodiazepines subsequently assayed in vitro (187).

This may be related to a similar increase in binding sites seen in the cerebral cortex assayed in vitro following electroshock or pentylenetetrazole seizures (187a) or in the hippocampus following amygdala kindling seizures (188). Increased binding was also observed at 1 hour following a single acute dose of benzodiazepines (189). Decreased binding was found after chronic high doses (100 mg/kg) of benzodiazepines for 1 week or longer (190), but not after 30 days at lower doses (3 mg/kg) (191), or after withdrawal from the drugs (192). No changes in benzodiazepine binding were seen following 7–10 days administration of 100 mg/kg barbital (190) or 30 days of 30 mg/kg phenobarbital (191), but a decrease in B_{max} was reported in the mouse following 4 days of 60 mg/kg phenobarbital (193). These studies indicate that benzodiazepine binding sites may be regulated by the excitatory state of the brain.

The block of barbiturate and etazolate enhancement of benzodiazepine binding by bicuculline suggests an indirect interaction between the barbiturate and GABA receptors. This idea is supported by numerous other observed interactions between the two receptors. Interestingly, although bicuculline completely reverses both GABA and etazolate enhancement of benzodiazepine binding, it only blocked pentobarbital enhancement partially (83), from 20–80%, the degree of which varied with brain region, cortex > hippocampus = striatum = thalamus > medulla-pons = cerebellum (194). This indicates a variable degree of coupling between barbiturate and GABA receptors, as well as a difference between pentobarbital and etazolate. The maximal effect of pentobarbital and etazolate on benzodiazepine binding also varied slightly with brain region, and not completely in parallel (194). Furthermore, the maximal effect of pentobarbital was always slightly higher than that of etazolate, and in the presence of both agents together, benzodiazepine binding was enhanced to the level of pentobarbital alone rather than to the lower maximum of etazolate as seen with partial agonists. This suggests the possible presence of a population of pentobarbital-enhanced benzodiazepine receptors which are not affected by etazolate or bicuculline (83).

BARBITURATE/PICROTOXININ-GABA RECEPTOR INTERACTIONS

Drugs that bind to barbiturate/picrotoxinin receptors also interact with GABA receptor sites. GABA agonists and etazolate mutually potentiate each other in enhancing benzodiazepine binding (89, 170); even GABA analogues, which do not enhance benzodiazepine binding at 0°C, will en-

hance the action of etazolate (89). The convulsant benzodiazepine RO5-3663 (164), which acts at barbiturate-picrotoxinin sites (105), inhibited the GABA enhancement of benzodiazepine binding (195), as did picrotoxinin and isopropyl bicyclophosphate, although only at 37° and/or at high Cl^- concentrations (196, 197). Pentobarbital was reported to enhance the ability of GABA to enhance benzodiazepine binding (198), at concentrations lower than those needed to enhance base line benzodiazepine binding in one study (172) but not in another (171). Etazolate, now known to act in a manner similar but not identical to pentobarbital in these in vitro systems (83, 170), caused a chloride-stimulated increase in GABA receptor binding apparently due to an increase in the number of sites B_{max} (199). This effect varied in intensity with brain region and was lost entirely upon treating the membranes with Triton X-100 under conditions known to improve GABA binding (199). Another depressant drug, etomidate, has been shown to cause an apparent increase in the number of high affinity GABA sites assayed in vitro (200).

Barbiturates have a similar effect on GABA receptor binding. Willow & Johnston (201) observed an enhancement in GABA binding affinity (from a K_D of about 89 to 55 nM) in the presence of 100 μM pentobarbital; this effect was thought to depend on a gentle membrane preparation, involving multiple washes but no osmotic shock, no tissuemizer treatment, no freezing and thawing, and above all, no detergent exposure. Tissuemizer disruption decreased the potency of pentobarbital. The effect of barbiturates on GABA binding was actually a biphasic one in which high concentrations of the drug (1 mM) reversed the enhancement of GABA binding seen at lower concentrations (202); such an effect has been described for the enhancement of benzodiazepine binding by etazolate (170) and certain barbiturates (175, 176). Treatment of the membranes with Triton X-100 abolished the enhancement but not the down-turn effect at high concentrations, which became an inhibition of GABA binding in the absence of the enhancement (202). The reversal effect was not characterized with respect to barbiturate specificity, but the enhancement of GABA binding was shown to occur with excitatory barbiturates such as CHEB and 5-ethyl, 5-(3-methyl but-2-enyl) barbiturate; typical hypnotic barbiturates such as secobarbital, butobarbital, amobarbital, and pentobarbital; and typical anticonvulsant barbiturates like phenobarbital (203). Although these authors did not observe any high affinity ($K_D \simeq 10$–20 nM) GABA binding in their first report (201), they did observe low affinity GABA binding ($K_D \simeq 1$ μM) which was not characterized as far as receptor specificity (nor was it affected by pentobarbital), in addition to the sites ($K_D \simeq 89$ nM) which were enhanced. Subsequently, they observed biphasic ligand dissociation kinetics (204) consistent with two-site binding curves (27, 96). They reported a slow

off-rate component ($k_{-1} = 0.17$ min^{-1}) accounting for part of their GABA binding, with the rest having a rate too fast to measure ($t_{½} < 5$ sec). The slowly dissociating component was decreased to $k_{-1} = 0.06$ min^{-1} in the presence of 100 μM pentobarbital (204). In a later report these authors (200) showed that pentobarbital lowered the K_D for a high affinity GABA binding site from 18 to 9 nM; they did not explain the differences between this result and their earlier work (201). Nevertheless, the observation of in vitro effects on GABA binding seems qualitatively very important. Quantitatively, a decreased dissociation rate would be consistent with an increased life-time for the receptor ligand complex, which could account for the increased life-time of GABA-activated Cl^- channels seen in the presence of pentobarbital (178).

Results from our laboratory agree in principle but not in detail with these observations. Whereas we found no effects of barbiturates on GABA binding in Tris-citrate buffer (34, 38), in the presence of Cl^- we found reproducible pentobarbital enhancement of GABA binding, using either fresh, osmotically shocked and dialyzed rat cortex membranes, or frozen and thawed, thoroughly washed tissuemizer-homogenized cow cortex membranes, but not Triton X-100-treated membranes (176, 205). We found the barbiturate enhancement to be dependent on the same anions [(176, 205); R. W. Olsen and A. Snowman, submitted] which, as described above, allow barbiturate-benzodiazepine interactions (171) and apparently correspond to anions able to permeate GABA ionophores (101). We also found that the pharmacological specificity for a series of barbiturates to enhance GABA binding was similar to that for enhancing benzodiazepine binding, including a lack of activity by phenobarbital but potent action by DMBB [(205); R. W. Olsen and A. Snowman, submitted].

In our hands, the in vitro barbiturate enhancement of GABA binding, like that of etazolate (199), appeared at first to be due to an increase in B_{max} (176, 205). Careful examination of multipoint binding curves with several radioactive GABA ligands led to the conclusion that a three-site fit to the data was as statistically suitable as the two-site fits previously described (27). This suggested that a low affinity GABA receptor binding site ($K_D = 1$ μM) might be present in addition to the two components ($K_D =$ 10–20 nM and $K_D =$ 100–200 nM) already described (27). The low affinity binding sites were most readily detected with the highest affinity ligand, [^{3}H] muscimol (R. W. Olsen and A. Snowman, submitted). As pointed out by others (206), under some conditions [^{3}H] muscimol gives an *apparent* greater B_{max} than the other ligands because it can detect more low affinity binding. The low affinity binding sites were enhanced in affinity by barbiturates, with accompanying decrease in binding dissociation rate (R. W. Olsen and A. Snowman, submitted).

The enhancement of GABA binding was variable with brain region in a manner which did not parallel overall GABA binding or low affinity (K_D = 100–200 nM) binding; the regional variation rather agreed with the variation in the bicuculline-sensitive portion of pentobarbital-enhanced benzodiazepine binding (194). This suggests that a considerable portion of GABA receptor sites which are not coupled to barbiturate receptors are present in some areas like cerebellum. Furthermore, considerable numbers of barbiturate receptors coupled to benzodiazepine receptors but not to GABA receptors exist in some brain regions, especially cerebellum. In this regard, etazolate resembled barbiturates, showing a quite variable enhancement of GABA binding with brain region and an especially low activity in cerebellum [(194); F. Leeb-Lundberg and R. W. Olsen, submitted].

DISCUSSION AND CONCLUSIONS

In vitro interactions between benzodiazepine, barbiturate, and GABA receptors are consistent with physiological observations that some of the actions of all of these drugs converge at the level of the GABA receptor-ionophore complex in mammalian brain. Quantitative considerations have been made difficult by the apparent presence in brain of unknown quantities of the three types of drug binding sites of interest which do *not* show mutual interactions. The extent to which this reflects either different coupling sites as outlined in Figure 1, or different receptor subtypes, some of which may not even be related to the GABA complex described, is unknown and will challenge future investigations. For example, bicuculline- and isoguvacine-insensitive GABA binding sites showing a high affinity for the anti-spastic drug baclofen have recently been defined (207). It may be valuable, however, to see how far we can get by applying simple models to the system.

Figure 2 shows a theoretical two-state model for the benzodiazepine receptor. The equilibrium between the two states would greatly favor the lower affinity state on the left, with significant energy barriers existing between the two states. Associated with the benzodiazepine receptor are the GABA receptor sites and barbiturate receptor sites. Occupancy of either of

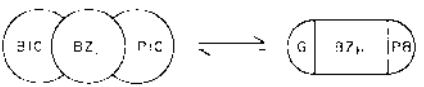

Figure 2 Two-state model of the benzodiazepine receptor. The benzodiazepine receptor (BZ) in coupled to the GABA receptor [labeled by the agonist GABA (G) or the antagonist bicuculline (BIC)] and the barbiturate receptors [labeled by the agonist pentobarbital (PB) or the antagonist picrotoxinin (PIC)].

these receptor sites by agonists will favor a shift to the high affinity benzodiazepine binding state on the right. This allosteric enhancement would result from any GABA agonist binding to its receptor, an effect blocked by antagonists like bicuculline, which favor the state on the left. A similar enhancement would result from barbiturate agonists binding to their receptor, an effect blocked by picrotoxinin and related convulsants. The effects on benzodiazepine binding of GABA and barbiturate ligands with full agonist properties would be additive at submaximal concentrations, but eventually nonadditive. Partial agonists acting at either GABA or barbiturate sites would produce an intermediate effect by binding to both states. Due to the presence somewhere in this complex of the chloride ionophore (Figure 1*A*), many of the interactions between ligands acting at the various receptors are either chloride-dependent or at least chloride-sensitive, with stronger interactions occurring at physiological concentrations. Other conditions which approximate as closely as possible the physiologically normal cellular environment (such as 37° temperatures), seem to maximize the interactions. This might be reason for hoping that the in vitro observations made under these conditions could provide information relevant to in vivo functioning of the system. This model suggests that bicuculline-like agents might reverse not only the GABA enhancement of benzodiazepine binding but also that of barbiturate-like substances (as observed), and that picrotoxinin-like drugs might reverse not only the enhancement by barbiturates but also that of GABA; this was observed but only in assays performed at 37° at high chloride concentrations (196). Furthermore, when binding was done on intact cultured rat cortex cells at 36°C (10), picrotoxinin reversed GABA enhancement of benzodiazepine binding in a chloride-sensitive manner. Interestingly, under these conditions (which might be considered in some ways to be the most physiological yet employed, despite certain obvious differences from the in situ situation), benzodiazepines bound to receptors with dose-response curves mimicking their potentiation of GABA responses measured electrophysiologically. In addition, GABA enhancement of benzodiazepine binding required rather high concentrations (> 10 μM) (similar to those required to activate Cl^- channels?), and benzodiazepine binding was *inhibited* by Cl^- ions, in a picrotoxinin-and GABA-modulated manner (10).

These observations, together with those outlined above, qualitatively support the existence of the three-receptor-ion channel complex as described in Figures 1*A* and 2. But what do they tell us about the functioning of the GABA receptor-ionophore and its modulation by drugs?

It is possible that the two conformational states in Figure 2 correspond to the resting and activated states of the GABA receptor-ionophore, but this remains to be demonstrated. From the interactions with benzodiazepines

and barbiturates it seems reasonable that one or both of the GABA receptor states shown in Figure 2 is related to the functional state which regulates Cl^- channels. The effect of barbiturates on GABA binding would be consistent with a low and high affinity state for GABA sites on the left and right. However, the benzodiazepine-GABA binding interactions suggest that both states of the GABA receptor involved would be relatively low affinity sites. Likewise, the physiological dose-response curves for GABA suggest that the resting and activated states of functioning GABA receptors would be low affinity, thus relegating the "high" and "low" affinity (K_D = 10–20 and 100–200 nM) sites measured in vitro to homogenization artifacts or perhaps inactive forms of the receptor such as a "desensitized" state, or one that is "uncoupled" from effectors including the Cl^- channel or modulating drug receptors. The evidence does seem to suggest that the GABA sites of K_D = 10–20 nM are not coupled to benzodiazepine receptors, although GABA binding to sites with K_D = 10–100 nM is increased by barbiturates, acting on sites with K_D = 100–1000 nM. These latter sites would seem to be involved in the complex which includes the modulatory drug receptors (Figures 1*A* and 2). The different effects of barbiturates and benzodiazepines on GABA binding might be related to their differences in mechanism at the GABA receptor-ionophore. Recent studies on fluctuation analysis in cultured spinal cord neurons indicate that barbiturates increase the lifetime of GABA-activated Cl^- channels, but that benzodiazepines increase the frequency of opening of GABA receptor regulated Cl^- channels (208). *If* the in vitro binding studies can be compared to the situation in the cell, one might expect an enhancement in GABA affinity by barbiturates but some other perturbation by benzodiazepines. For the moment, however, the in vitro interactions between GABA and the other drugs are merely evidence for the existence of complexes as in Figure 1, and we will be very fortunate if they tell us anything about mechanism in vivo.

With respect to the multiple forms of receptor binding and possible coupling states (209), some correlations might be made. For example, the high levels of high affinity GABA binding in cerebellum which are not coupled to benzodiazepine binding indicate a considerable amount of the GABA receptor in form *C* or *E* (Figure 1). Poor barbiturate enhancement of GABA binding in cerebellum might indicate that *C* is also low, so *E* is high. Cerebellum also shows a high level of bicuculline-insensitive, pentobarbital-enhanced benzodiazepine binding, and therefore a relatively high ratio of form *D* over *A*. Benzodiazepine binding and CL 218,472 binding are reasonably well enhanced in cerebellum, but this could be due to form *B* if form *A* is present in low amounts. It seems reasonable that the "cerebellum-type" of benzodiazepine receptor might also be represented by one of the forms in Figure 1, and if so, it would not be form *A,* but *B* or *D*. Thus,

current biochemical and anatomical studies are beginning to clarify the nature and composition of multiple receptor binding sites in various brain regions which should be useful in understanding what appears today to be a very complicated situation.

ACKNOWLEDGMENTS

This research was supported by grants from the National Institutes of Health, including a Research Career Development Award from the NINCDS, from the National Science Foundation and from Hoffmann-La Roche, Inc. I thank A. Snowman and F. Leeb-Lundberg for participating in this research project, N. Birdsall, E. Hulme, J. Barker, and J. Cohen for helpful discussions, and N. Carwile for typing the manuscript.

Literature Cited

1. McBurney, R. N., Barker, J. L. 1978. GABA-induced conductance fluctuations in cultured spinal neurones. *Nature* 274:596–97
2. Nistri, A., Constanti, A., Krnjević, K. 1980. Electrophysiological studies of the mode of action of GABA on vertebrate central neurons. *Adv. Biochem. Psychopharmacol.* 21:81–90
3. Changeux, J. P. 1981. The acetylcholine receptor: an allosteric membrane protein. *Harvey Lect.* In press
4. Haefely, W., Polc, P., Schaffner, R., Keller, H. H., Pieri, L., Möhler, H. 1979. Facilitation of GABA-ergic transmission by drugs. In *GABA-Neurotransmitters,* ed. P. Krogsgaard-Larsen, J. Scheel-Kruger, H. Kofod, pp. 357–75. Copenhagen: Munksgaard 552 pp.
5. Olsen, R. W. 1981. GABA-benzodiazepine-barbiturate receptor interactions. *J. Neurochem.* 37:1–13
6. Simmonds, M. A. 1980. A site for the potentiation of GABA-mediated responses by benzodiazepines. *Nature* 284:558–60
7. Choi, D. W., Farb, D. H., Fischbach, G. D. 1977. Chlordiazepoxide selectively augments GABA action in spinal cord cell cultures. *Nature* 269:342–44
8. Macdonald, R. L., Barker, J. L. 1978. Benzodiazepines specifically modulate GABA-mediated postsynaptic inhibition in cultured mammalian neurons. *Nature* 271:563–64
9. Mitchell, R. 1980. Benzodiazepines modify the agonist responses at a presynaptic GABA receptors. *Eur. J. Pharmacol.* 68:369–72
10. White, W. F., Dichter, M. A., Snodgrass, S. R. 1981 Benzodiazepine binding and interactions with the GABA receptor complex in living cultures of rat cerebral cortex. *Brain Res.* 215: 162–76
11. Simmonds, M. A. 1981. Distinction between the effects of barbiturates, benzodiazepines and phenytoin on responses to γ-aminobutyric acid receptor activation and antagonism by bicuculline and picrotoxinin. *Br. J. Pharmacol.* 73:739–47
12. Macdonald, R. L., Barker, J. L. 1978. Different actions of anticonvulsant and anesthetic barbiturates revealed by use of cultured mammalian neurons. *Science* 200:775–77
13. Nicoll, R. A., Eccles, J. C., Oshima, T. C., Rubia, F. 1975. Prolongation of hippocampal inhibitory post-synaptic potentials by barbiturates. *Nature* 258: 625–27
14. Bowery, N. G., Dray, A. 1978. Reversal of the action of amino acid antagonists by barbiturates and other hypnotic drugs. *Br. J. Pharmacol.* 63:179–215
15. Brown, D. A., Constanti, A. 1978. Interaction of pentobarbitone and γ-aminobutyric acid on mammalian sympathetic ganglion cells. *Br. J. Pharmacol.* 63:217–24
16. Evans, R. H. 1979. Potentiation of the effects of GABA by pentobarbitone. *Brain Res.* 171:113–20
17. Schulz, D. W., Macdonald, R. L. 1981. Barbiturate enhancement of GABA-mediated inhibition and activation of chloride ion conductance: correlation with anticonvulsant and anesthetic actions. *Brain Res.* 209:177–88
18. Curtis, D. R., Lodge, D. 1977. Pentobarbitone enhancement of the inhibi-

tory action of GABA. *Nature* 270:543–44
19. Andrews, P. R., Johnston, G. A. R. 1979. GABA agonists and antagonists. *Biochem. Pharmacol.* 28:2697–2702
20. Zukin, S. R., Young, A. B., Snyder, S. H. 1974. Gamma-aminobutyric acid binding to receptor sites in the rat central nervous system. *Proc. Natl. Acad. Sci. USA.* 71:4802–7
21. Krogsgaard-Larsen, P., Hjeds, H., Curtis, D. R., Lodge, D., Johnston, G. A. R. 1979. Dihydromuscimol, thiomuscimol and related heterocyclic compounds as GABA analogues. *J. Neurochem.* 32:1717–24
22. Enna, S. J., Snyder, S. H. 1975. Properties of γ-aminobutyric acid (GABA) receptor binding in rat brain synaptic membrane fractions. *Brain Res.* 100:81–97
23. Greenlee, D. V., Van Ness, P. C., Olsen, R. W. 1978. Endogenous inhibitor of GABA binding in mammalian brain. *Life Sci.* 22:1653–62
24. Napias, C., Bergman, M. O., Van Ness, P. C., Greenlee, D. V., Olsen, R. W. 1980. GABA binding in mammalian brain: inhibition by endogenous GABA. *Life Sci.* 27:1001–11
25. Coyle, J. T., Enna, S. J. 1976. Neurochemical aspects of the ontogenesis of GABAnergic neurons in the rat brain. *Brain Res.* 111:119–33
26. Greenlee, D. V., Van Ness, P. C., Olsen, R. W. 1978. Gamma-aminobutyric acid binding in mammalian brain: receptor-like specificity of sodium-independent sites. *J. Neurochem.* 31:933–38
27. Olsen, R. W., Bergman, M. O., Van Ness, P. C., Lummis, S. C., Watkins, A. E., Napias, C., Greenlee, D. V. 1981. γ-Aminobutyric acid receptor binding in mammalian brain: heterogeneity of binding sites. *Mol. Pharmacol.* 19: 217–27
28. Van Ness, P. C., Watkins, A. E., Bergman, M. O., Tourtellotte, W. W., Olsen, R. W. 1982. γ-Aminobutyric acid receptors in normal human brain and Huntington's disease. *Neurology.* In press
29. Beaumont, K., Chilton, W., Yamamura, H. I., Enna, S. J. 1978. Muscimol binding in rat brain: association with synaptic GABA receptors. *Brain Res.* 148:153–62
30. Wang, Y.-J., Salvaterra, P., Roberts, E. 1979. Characterization of ^{3}H-muscimol binding to mouse brain membranes. *Biochem. Pharmacol.* 28:1123–28
31. Greenlee, D. V., Olsen, R. W. 1979. Solubilization of gamma-aminobutyric acid receptor protein from mammalian brain. *Biochem. Biophys. Res. Commun.* 88:380–87
32. Morin, A. M., Wasterlain, C. 1980. The binding of ^{3}H-isoguvacine to mouse brain synaptic membranes. *Life Sci.* 26:1239–45
33. Krogsgaard-Larsen, P., Snowman, A., Lummis, S. C., Olsen, R. W. 1981. Characterization of the binding of the GABA agonist [^{3}H] piperidine-4-sulphonic acid (P4S) to bovine brain synaptic membranes. *J. Neurochem.* 37:401–9
34. Olsen, R. W. 1980. Biochemistry of GABA receptor proteins in mammalian brain. *Psychopharmacology and Biochemistry of Neurotransmitter Receptors,* ed. H. I. Yamamura, R. W. Olsen, E. Usdin, pp. 537–50. New York: Elsevier: 676 pp.
35. Enna, S. J. 1981. GABA receptor pharmacology: functional considerations. *Biochem. Pharmacol.* 30:907–13
36. Krogsgaard-Larsen, P., Falch, E. 1981. GABA agonists: development and interactions with the GABA receptor complex. *Mol. Cell. Biochem.* In press
37. Johnston, G. A. R. 1981. GABA receptors. In *The Role of Peptides and Amino Acids as Neurotransmitters,* New York: Liss. In press
38. Olsen, R. W., Ticku, M. K., Van Ness, P. C., Greenlee, D. 1978. Effects of drugs on γ-aminobutyric acid receptors, uptake, release and synthesis *in vitro. Brain Res.* 139:277–94
39. Leeb-Lundberg, F., Olsen, R. W. 1980. Picrotoxinin binding as a probe of the GABA postsynaptic membrane receptor-ionophore complex. See Ref. 34, pp. 593–606
40. Guidotti, A., Toffano, G., Costa, E. 1978. An endogenous protein modulates the affinity of GABA and benzodiazepine receptors in rat brain. *Nature* 275:553–55
41. Costa, E., Guidotti, A. 1979. Molecular mechanisms in the receptor action of benzodiazepines. *Ann. Rev. Pharmacol. Toxicol.* 19:531–45
42. Toffano, G., Guidotti, A., Costa, E. 1978. Purification of an endogenous protein inhibitor of the high affinity binding of γ-aminobutyric acid to synaptic membranes of rat brain. *Proc. Natl. Acad. Sci. USA* 75:4024–28
43. Massotti, M., Guidotti, A. 1980. Endogenous regulators of benzodiazepine recognition sites. *Life Sci.* 27:847–54

44. Massotti, M., Guidotti, A., Costa, E. 1981. Characterization of benzodiazepine and γ-aminobutyric acid recognition sites and their endogenous modulators. *J. Neurosci.* 1:409–18
45. Tallman, J. F., Paul, S. M., Skolnick, P., Gallager, D. W. 1980. Receptors for the age of anxiety: pharmacology of the benzodiazepines. *Science* 207:274–81
46. Gavish, M., Snyder, S. H. 1980. Benzodiazepine recognition sites on GABA receptors. *Nature* 287:651–52
47. Squires, R. F., Braestrup, C. 1977. Benzodiazepine receptors in rat brain. *Nature* 266:732–34
48. Möhler, H., Okada, T. 1977. Benzodiazepine receptors: demonstration in the central nervous system. *Science* 198:849–51
49. Bosmann, H. B., Case, K. R., Di Stefano, P. 1977. Diazepam receptor characterization: specific binding of a benzodiazepine to macromolecules in various areas of rat brain. *FEBS Lett.* 82:368–72
50. Müller, W. E., Schlafer, U., Wollert, U. 1978. Benzodiazepine receptor binding: the interaction of some non-benzodiazepine drugs with specific [^{3}H]diazepam binding to rat brain synaptosomal membranes. *Naunyn-Schmeidebergs Arch. Pharmacol.* 305:23–26
51. Mackerer, C. R., Kochman, R. L., Bierschenck, B. A., Bremner, S. S. 1978. The binding of [^{3}H] diazepam to rat brain homogenates. *J. Pharmacol. Exp. Ther.* 206:405–13
52. Braestrup, C., Squires, R. F. 1977. Specific benzodiazepine receptors in rat brain characterized by high affinity [^{3}H] diazepam binding. *Proc. Natl. Acad. Sci. USA* 74:3805–9
53. Wastek, G. J., Speth, R. C., Reisine, T. D., Yamamura, H. I. 1978. The effect of gamma-aminobutyric acid on ^{3}H-flunitrazepam binding in the rat brain. *Eur. J. Pharmacol.* 50:445–47
54. Bosmann, H. B., Penney, D. P., Case, K. R., DiStefano, P., Averill, K. 1978. Diazepam receptor: specific binding of [^{3}H]flunitrazepam to rat brain subfractions. *FEBS Lett.* 87:199–202
55. Speth, R. C., Wastek, G. J., Johnson, P. C., Yamamura, H. I. 1978. Benzodiazepine binding in human brain: characterization using [^{3}H]flunitrazepam. *Life Sci.* 22:859–66
56. Möhler, H., Battersby, M. K., Richards, J. G. 1980. Benzodiazepine receptor protein identified and visualized in brain tissue by a photoaffinity label. *Proc. Natl. Acad. Sci. USA* 77:1666–70
57. Sieghart, W., Karobath, M. 1980. Molecular heterogeneity of benzodiazepine receptors. *Nature* 286:285–87
58. Syapin, P. J., Skolnick, P. 1979. Characterization of benzodiazepine binding sites in cultured cells of neural origin. *J. Neurochem.* 32:1047–51
59. McCarthy, K.D., Harden, T.K. 1981. Identification of two benzodiazepine binding sites on cells cultured from rat cerebral cortex. *J. Pharmacol. Exp. Ther.* 216:183–91
60. Huang, A., Barker, J. L., Paul, S. M., Moncada, V. M., Skolnick, P. 1980. Characterization of benzodiazepine receptors in primary cultures of fetal mouse brain and spinal cord neurons. *Brain Res.* 190:485–91
61. Braestrup, C., Nissen, C., Squires, R. F., Schousboe, A. 1978. Lack of brain specific benzodiazepine receptors in mouse primary astroglial cultures. *Neurosci. Lett.* 9:45–9
62. Yokoi, I., Rose, S. E., Yanagihara, T. 1981. Benzodiazepine receptor: heterogeneity in rabbit brain. *Life Sci.* 28:1591–95
63. Schoemaker, H., Bliss, M., Yamamura, H. I. 1981. Specific high-affinity saturable binding of [^{3}H]RO5-4864 to benzodiazepine binding sites in the rat cerebral cortex. *Eur. J. Pharmacol.* 71: 173–75
64. Squires, R. F., Benson, D. I., Braestrup, C., Coupet, J., Klepner, C. A., Myers, V., Beer, B. 1979. Some properties of brain specific benzodiazepine receptor: new evidence for multiple receptors. *Pharmacol. Biochem. Behav.* 10:825–30
65. Braestrup, C., Nielsen, M., Olsen, C. E. 1980. Urinary and brain β-carboline-3-carboxylates as potent inhibitors of brain benzodiazepine receptors. *Proc. Natl. Acad. Sci. USA* 77:2288–92
66. Regan, J. W., Roeske, W. R., Malick, J. B., Yamamura, S. H., Yamamura, Y. I. 1981. GABA enhancement of CL 218, 872 affinity and evidence of benzodiazepine receptor heterogeneity. *Mol. Pharmacol.* 20:477–83
67. Yamamura, H. I., Mimaki, T., Morelli, M., Yamamura, S. H., Horst, W. D., Bautz, G., O'Brien, R. 1981. A novel triazolopyridazine, ^{3}H-CL 218,872 which labels the benzodiazepine receptor in rat brain. In *Protides of the Biological Fluids 29,* ed. H. Peeters. New York: Pergamon. Vol. 29: In press
68. Young, W. S. III, Niehoff, D., Kuhar, M. J., Beer, B., Lippa, A. S. 1981. Multiple benzodiazepine receptor localization by light microscopic radiohisto-

chemistry. *J. Pharmacol. Exp. Ther.* 216:425–30
69. Lippa, A. S., Beer, B., Sano, M. C., Vogel, R. A., Meyerson, L. R. 1981. Differential ontogeny of type 1 and type 2 benzodiazepine receptors. *Life Sci.* 28:2343–47
70. Nielsen, M., Braestrup, C. 1980. Ethyl-β-carboline-3-carboxylate shows differential benzodiazepine receptor interaction. *Nature* 286:606–7
71. Nielsen, M., Schou, H., Braestrup, C. 1981. [^{3}H] Propyl β-carboline-3-carboxylate binds specifically to brain benzodiazepine receptors. *J. Neurochem.* 36:276–85
72. Williams, E. F., Rice, K. C., Paul, S. M., Skolnick, P. 1980. Heterogeneity of benzodiazepine receptors in the central nervous system demonstrated with kenazepine, an alkylating benzodiazepine. *J. Neurochem.* 35:591–97
73. Squires, R. F. 1981. GABA receptors regulate the affinities of anions required for brain specific benzodiazepine binding. *Adv. Biochem. Psychopharmacol.* 26:129–38
74. Gallager, D. W., Thomas, J. W., Tallman, J. F. 1978. Effect of GABAergic drugs on benzodiazepine binding site sensitivity in rat cerebral cortex. *Biochem. Pharmacol.* 27:2745–49
75. Tallman, J. F., Thomas, J. W., Gallager, D. W. 1978. GABAergic modulation of benzodiazepine binding site sensitivity. *Nature* 274:383–85
76. Briley, M. S., Langer, S. Z. 1978. Influence of GABA receptor agonists and antagonists on the binding of ^{3}H-diazepam to the benzodiazepine receptor. *Eur. J. Pharmacol.* 52:129–32
77. Martin, I. L., Candy, J. M. 1978. Facilitation of benzodiazepine binding by sodium chloride and GABA. *Neuropharmacology* 17:993–98
78. Karobath, M., Sperk, G. 1979. Stimulation of benzodiazepine receptor binding by gamma-aminobutyric acid. *Proc. Natl. Acad. Sci. USA* 76:1004–6
79. Dudai, Y. 1979. Modulation of benzodiazepine binding sites in calf cortex by an endogenous factor and GABAergic ligands. *Brain Res.* 167:422–25
80. Chiu, T. H., Rosenberg, H. C. 1979. GABA receptor-mediated modulation of ^{3}H-diazepam binding in rat cortex. *Eur. J. Pharmacol.* 56:337–45
81. Young, W. S., Kuhar, M. J. 1979. Autoradiographic localisation of benzodiazepine receptors in the brains of humans and animals. *Nature* 280: 393–95
82. Braestrup, C., Nielsen, M., Krogsgaard-Larsen, P., Falch, E. 1979. Partial agonists for brain GABA/benzodiazepine receptor complex. *Nature* 280:331–33
83. Leeb-Lundberg, F., Snowman, A., Olsen, R. W. 1981. Perturbation of benzodiazepine receptor binding by pyrazolpyridines involves picrotoxinin/barbiturate receptor sites. *J. Neurosci.* 1:471–77
84. Gavish, M., Snyder, S. H. 1980. Soluble benzodiazepine receptors: GABAergic regulation. *Life Sci.* 26:579–82
85. Karobath, M., Placheta, P., Lippitsch, M., Krogsgaard-Larsen, P. 1979. Is stimulation of benzodiazepine receptor binding mediated by a novel GABA receptor? *Nature* 278:748–49
86. Maurer, R. 1979. The GABA agonist THIP, a muscimol analogue, does not interfere with the benzodiazepine binding site on rats cortical membranes. *Neurosci. Lett.* 12:65–68
87. Karobath, M., Lippitsch, M. 1979. THIP and isoguvacine are partial agonists of GABA-stimulated benzodiazepine receptor binding. *Eur. J. Pharmacol.* 58:485–88
88. Supavilai, P., Karobath, M. 1980. The effect of temperature and chloride ions on the stimulation of [^{3}H] flunitrazepam binding by the muscimol analogues THIP and piperidine-4-sulfonic acid. *Neurosci. Lett.* 19:337–41
89. Supavilai, P., Karobath, M. 1980. Interaction of SQ 20009 and GABA-like drugs as modulators of benzodiazepine receptor binding. *Eur. J. Pharmacol.* 62:229–33
90. Supavilai, P., Karobath, M. 1980. Heterogeneity of benzodiazepine receptors in rat cerebellum and hippocampus. *Eur. J. Pharmacol.* 64:91–93
91. Maggi, A., Satinover, J., Oberdorfer, M., Mann, E., Enna, S. J. 1980. Phylogenetic characteristics of muscimol-activated benzodiazepine receptor binding. *Brain Res. Bull.* 5:Suppl. 2, pp. 167–71
92. Asano, T., Ogasawara, N. 1981. Soluble gamma-aminobutyric acid and benzodiazepine receptors from rat cerebral cortex. *Life Sci.* 29:193–200
93. Stone, M. A., Topham, L. D. 1980. Does binding to the high and low affinity GABA binding sites represent binding to benzodiazepine receptor linked and non-benzodiazepinereceptor linked GABA receptors? *Br. J. Pharmacol.* 69:326P-27P

94. Barker, J. L., Mathers, D. A. 1981. GABA analogues activate channels of different duration on cultured mouse spinal neurons. *Science* 212:358–60
95. Enna, S. J., Snyder, S. H. 1977. Influences of ions, enzyme, and detergents on γ-aminobutyric acid-receptor binding in synaptic membranes of rat brain. *Mol. Pharmacol.* 13:442–53
96. Browner, M., Ferkany, J. W., Enna, S. J. 1981. Biochemical identification of pharmacologically and functionally distinct GABA receptors in rat brain. *J. Neurosci.* 1:514–18
97. Mackerer, C. R. Kochman, R. L. 1978. Effects of cations and anions on the binding of ^{3}H-diazepam to rat brain. *Proc. Soc. Exp. Biol. Med.* 158:393–97
98. Costa, T., Rodbard, D., Pert, C. B. 1979. Is the benzodiazepine receptor coupled to a chloride anion channel? *Nature* 277:315–17
99. Ehlert, F. J., Roeske, W. R., Braestrup, C., Yamamura, S. H., Yamamura, H. I. 1981. γ-Aminobutyric acid regulation of the benzodiazepine receptor: biochemical evidence for pharmacologically different effects of benzodiazepines and propyl β-carboline-3-carboxylate. *Eur. J. Pharmacol.* 70:593–96
100. Araki, T., Ito, M., Oscarsson, O. 1961. Anion permeability of the synaptic and nonsynaptic motoneurone membrane. *J. Physiol.* 159:410–35
101. Eccles, J., Nicoll, R. A., Oshima, T., Rubia, F. J. 1977. The anionic permeability of the inhibitory postsynaptic membrane of hippocampal pyramidal cells. *Proc. R. Soc. London Ser. B.* 198:345–61
102. Martin, I. L., Candy, J. M. 1980. Facilitation of specific benzodiazepine binding in rat brain membrane fragments by a number of anions. *Neuropharmacology* 19:175–79
103. Kelly, J. S., Krnjević, K., Morris, M. E., Yim, G. K. W. 1969. Anionic permeability of cortical neurones. *Exp. Brain. Res.* 7:11–31
104. Lummis, S. C. R., Olsen, R. W. 1982. GABA receptor binding in mammalian brain: two sites, one specificity. Unpublished data
105. Løeb-Lundberg, F., Napias, C., Olsen, R. W. 1981. Dihydropicrotoxinin binding sites in mammalian brain: interactions with convulsant and depressant benzodiazepines. *Brain Res.* 216:399–408
106. Placheta, P., Karobath, M. 1979. Regional distribution of Na^+-independent GABA and benzodiazepine binding sites in rat CNS. *Brain Res.* 178:580–83
107. Palacios, J. M., Wamsley, J. K., Kuhar, M. J. 1981. GABA, benzodiazepine and histamine-H_1 receptors in the guinea pig cerebellum: effects of kainic acid injections studied by autoradiographic methods. *Brain Res.* 214:155–62
108. Meyerson, L. R., Sano, M. C., Critchett, D. J., Beer, B., Lippa, A. S. 1981. Lateral olfactory tract lesions reveal neuronal localization of benzodiazepine recognition sites. *Eur. J. Pharmacol.* 71:147–50
109. Speth, R. C., Bresolin, N., Mimaki, T., Deshmukh, P. P., Yamamura, H. I. 1981. Neuronal localization of benzodiazepine receptors in the murine cerebellum. *Adv. Biochem. Psychopharmacol.* 26:27–39
110. DeFeudis, F. V., Ossola, L., Schmitt, G., Wolff, P., Mandel, P. 1980. Na^+-independent binding of [^{3}H] GABA and [^{3}H] muscimol to subcellular particles of neural primary cultures and whole brain. *J. Neurochem.* 34:216–18
111. Napias, C., Olsen, R. W., Schubert, D. 1980. GABA and picrotoxinin receptors in clonal nerve cells. *Nature* 283:298–99
112. Ticku, M. K., Huang, A., Barker, J. L. 1980. GABA receptor binding in cultured mammalian spinal cord neurons. *Brain Res.* 182:201–6
113. Howells, R. D., Simon, E. J. 1980. Benzodiazepine binding in chicken retina and its interaction with γ-aminobutyric acid. *Eur. J. Pharmacol.* 67:133–37
114. Regan, J. W., Roeske, W. R., Yamamura, H. I. 1980. [^{3}H] Flunitrazepam binding to bovine retina and the effect of GABA thereon. *Neuropharmacology* 19:413–14
115. Skolnick, P., Paul, S., Zatz, M., Eskay, R. 1980. Brain-specific benzodiazepine receptors are located in the inner plexiform layer of rat retina. *Eur. J. Pharmacol.* 66:133–36
116. Borbe, H. O., Müller, W. E., Wollert, U. 1980. The identification of benzodiazepine receptors with brain-like specificity in bovine retina. *Brain Res.* 182:466–69
117. Willow, M., Morgan, I. G. 1980. Retinal benzodiazepine receptors are destroyed by kainic acid lesions. *Neurosci. Lett.* 20:147–52
118. Yazulla, S., Brecha, N. 1981. Localized binding of [^{3}H] muscimol to synapses in chicken retina. *Proc. Natl. Acad. Sci. USA* 78:643–47
119. Osborne, N. N. 1980. Benzodiazepine

binding to bovine retina. *Neurosci. Lett.* 16:167–70
120. Guarneri, P., Corda, M. G., Concas, A., Biggio, G. 1981. Kainic acid-induced lesion of rat retina: differential effect on cyclic GMP and benzodiazepine and GABA receptors. *Brain Res.* 209: 216–20
121. Campochiaro, P., Schwarcz, R., Coyle, J. T. 1977. GABA receptor binding in rat striatum: localization and effects of denervation. *Brain Res.* 136:501–11
122. Sperk, G., Schlögl, E. 1979. Reduction of number of benzodiazepine binding sites in the caudate nucleus of the rat after kainic acid injections. *Brain Res.* 170:563–67
123. Chang, R. S., Tran, V. T., Snyder, S. H. 1980. Neurotransmitter receptor localizations: brain lesion induced alterations in benzodiazepine, GABA, beta-adrenergic and histamine H1-receptor binding. *Brain Res.* 190:95–110
124. Waddington, J. L., Cross, A. J. 1978. Denervation supersensitivity in the striatonigral GABA pathway. *Nature* 276:618–70
125. Guidotti, A., Gale, K., Suria, A., Toffano, G. 1979. Biochemical evidence for two classes of GABA receptors in rat brain. *Brain Res.* 172:566–71
126. Biggio, G., Corda, M. G., Lamberti, C., Gessa, G. L. 1979. Changes in benzodiazepine receptors following GABAergic denervation of substantia nigra. *Eur. J. Pharmacol.* 58:215–16
127. Shibuya, H., Gale, K., Pert, C. B. 1980. Supersensitivity to GABA's effect on benzodiazepine receptors develops after striatonigral lesions. *Eur. J. Pharmacol.* 62:243–44
128. Simantov, R., Oster-Granite, M. L., Herndon, R. M., Snyder, S. H. 1976. Gamma-aminobutyric acid (GABA) receptor binding selectively depleted by viral induced granule cell loss in hamster cerebellum. *Brain Res.* 105:365–71
129. Biggio, G., Corda, M. G., De Montis, G., Stefanini, E., Gessa, G. L. 1980. Kainic acid differentiates GABA receptors from benzodiazepine receptors in the rat cerebellum. *Brain Res.* 193:589–93
130. Olsen, R. W., Mikoshiba, K. 1978. Localization of gamma-aminobutyric acid receptor binding in the mammalian cerebellum: high levels in granule layer and depletion in agranular cerebella of mutant mice. *J. Neurochem.* 30: 1633–36
131. Skolnick, P., Syapin, P. J., Paugh, B. A., Paul, S. M. 1979. Reduction in benzodiazepine receptors associated with Purkinje cell degeneration in "nervous" mutant mice. *Nature* 277:397–99
132. Lippa, A. S., Sano, M. C., Coupet, J., Klepner, C. A., Beer, B. 1978. Evidence that benzodiazepine receptors reside on cerebellar Purkinje cells: studies with "nervous" mutant mice. *Life Sci.* 23:2213–18
133. Unnerstall, J. R., Kuhar, M. J., Niehoff, D. L., Palacios, J. M. 1981. Benzodiazepine receptors may be coupled to a subpopulation of GABA receptors: evidence from a quantitative autoradiographic study. *J. Pharmacol. Exp. Ther.* In press
134. Palacios, J. M., Young, W. S., Kuhar, M. J. 1980. Autoradiographic localization of γ-aminobutyric acid (GABA) receptors in the rat cerebellum. *Proc. Natl. Acad. Sci. USA* 77:670–74
135. Candy, J. M., Martin, I. L. 1979. The postnatal development of the benzodiazepine receptor in the cerebral cortex and cerebellum of the rat. *J. Neurochem.* 32:655–58
136. Braestrup, C., Nielsen, M. 1978. Ontogenetic development of benzodiazepine receptors in the rat brain. *Brain Res.* 147:170–73
137. Mallorga, P., Hamburg, M., Tallman, J. F., Gallager, D. W. 1980. Ontogenetic changes in GABA modulation of brain benzodiazepine binding. *Neuropharmacology* 19:405–8
138. Palacios, J. M., Niehoff, D. L., Kuhar, M. J. 1980. Ontogeny of GABA and benzodiazepine receptors: effects of Triton X-100, bromide and muscimol. *Brain Res.* 179:390–95
139. Regan, J. W., Roeske, W. R., Yamamura, H. I. 1980. The benzodiazepine receptor: its development and its modulation by γ-aminobutyric acid. *J. Pharmacol. Exptl. Ther.* 212:137–43
140. Möhler, H., Richards, J. G., Wu, J.-Y. 1981. Autoradiographic localization of benzodiazepine receptors in immunocytochemically identified γ-aminobutyrergic synapses. *Proc. Natl. Acad. Sci. USA* 78:1935–38
141. Chude, O. 1979. Solubilization and partial purification of the GABA receptor from mouse brain and a binding assay for the solubilized receptor. *J. Neurochem.* 33:621–29
142. Gavish, M., Chang, R. S. L., Snyder, S. H. 1979. Solubilization of histamine H-1, GABA and benzodiazepine receptors. *Life Sci.* 25:783–90

143. Lang, B., Barnard, E. A., Chang, L. R., Dolly, J. O. 1979. Putative benzodiazepine receptor: a protein solubilised from brain. *FEBS Lett.* 104:149–53
144. Yousufi, M. A. K., Thomas, J. W., Tallman, J. F. 1979. Solubilization of benzodiazepine binding site from rat cortex. *Life Sci.* 25:463–70
145. Chiu, T. H., Rosenberg, H. C. 1979. Differential effects of Triton X-100 on benzodiazepine and GABA binding in a frozen-thawed synaptosomal fraction of rat brain. *Eur. J. Pharmacol.* 58:335–38
146. Stephenson, F. A., Watkins, A. E., Olsen, R. W. 1981. Physicochemical characterization of detergent-solubilized γ-aminobutyric acid and benzodiazepine receptor proteins from bovine brain. *Eur. J. Biochem.* In press
147. Ticku, M. K., Davis, W. C. 1981. Evidence that ethanol and pentobarbital enhance [^{3}H]-diazepam binding at the benzodiazepine-GABA receptor-ionophore complex indirectly. *Eur. J. Pharmacol.* 71:521–22
148. Asano, T., Ogasawara, N. 1980. Solubilization of the benzodiazepine receptor from rat brain. *Life Sci.* 26:607–13
149. Sherman-Gold, R., Dudai, Y. 1980. Solubilization and properties of a benzodiazepine receptor from calf cortex. *Brain Res.* 198:485–90
150. Gavish, M., Snyder, S. H. 1981. γ-Aminobutyric acid and benzodiazepine receptors: copurification and characterization. *Proc. Natl. Acad. Sci. USA* 78:1939–42
151. Asano, T., Ogasawara, N. 1980. Solubilization of γ-aminobutyric acid receptor from rat brain. *Life Sci.* 26.1131–37
152. Chang, L.-R., Barnard, E. A., Lo, M. M. S., Dolly, J. O. 1981. Molecular sizes of benzodiazepine receptors and the interacting GABA receptors in the membrane are identical. *FEBS Lett.* 126:309–12
153. Ticku, M. K., Olsen, R. W. 1978. Interaction of barbiturates with dihydropicrotoxinin binding sites related to the GABA receptor-ionophore system. *Life Sci.* 22:1643–51
154. Ticku, M. K., Ban, M., Olsen, R. W. 1978. Binding of [^{3}H]-α-dihydropicrotoxinin, a gamma-aminobutyric acid synaptic antagonist, to rat brain membranes. *Mol. Pharmacol.* 14:391–402
155. Ticku, M. K., Olsen, R. W. 1979. Cage convulsants inhibit picrotoxinin binding. *Neuropharmacology* 18:315–18
156. Olsen, R. W., Ticku, M. K., Miller, T. 1978. Dihydropicrotoxinin binding to crayfish muscle sites possibly related to γ-aminobutyric acid receptor-ionophores. *Mol. Pharmacol.* 14:381–90
157. Ticku, M. K., Van Ness, P. C., Haycock, J. W., Levy, W. B., Olsen, R. W. 1978. Dihydropicrotoxinin binding sites in rat brain: comparison to GABA receptors. *Brain Res* 150:642–47
158. Olsen, R. W., Leeb-Lundberg, F. 1980. Endogenous inhibitors of picrotoxinin-convulsant binding sites in rat brain. *Eur. J. Pharmacol.* 65:101–4
159. Asano, T., Spector, S. 1979. Identification of inosine and hypoxanthine as endogenous ligands for the brain benzodiazepine-binding sites. *Proc. Natl. Acad. Sci. USA* 76:977–81
160. Marangos, P. J., Paul, S. M., Parma, A. M., Goodwin, F. K., Syapin, P., Skolnick, P. 1979. Purinergic inhibition of diazepam binding to rat brain (*in vitro.*) *Life Sci.* 24:851–58
161. Damm, H. W., Müller, W. E., Wollert, U. 1979. Is the benzodiazepine receptor purinergic? *Eur. J. Pharmacol.* 55: 331–33
162. Möhler, H., Polc, P., Cumin, R., Pieri, L., Kettler, R. 1979. Nicotinamide is a brain constituent with benzodiazepine-like actions. *Nature* 278:563–65
163. Ticku, M. K., Burch, T. 1980. Purine inhibition of [^{3}H] γ-aminobutyric acid receptor binding to rat brain membranes. *Biochem. Pharmacol.* 29: 1217–20
164. Schlosser, W., Franco, S. 1979. Reduction of gamma-aminobutyric acid (GABA)-mediated transmission by a convulsant benzodiazepine. *J. Pharmacol. Exp. Ther.* 211:290–95
165. Leeb-Lundberg, F., Snowman, A., Olsen, R. W. 1981. Interaction of anticonvulsants with the barbiturate/benzodiazepine/GABA receptor complex. *Eur. J. Pharmacol.* 72:125–29
166. Williams, M., Risley, E. A. 1979. Enhancement of the binding of ^{3}H-diazepam to rat brain membranes in vitro by SQ 20009, a novel anxiolytic, gamma-aminobutyric acid (GABA) and muscimol. *Life Sci.* 24:833–41
167. Beer, B., Klepner, C. A., Lippa, A. S., Squires, R. F. 1978. Enhancement of ^{3}H-diazepam binding by SQ65396: a novel anti-anxiety agent. *Pharmacol. Biochem. Behav.* 9:849–51
168. Salama, A. I., Meiners, B. A. 1980. Enhancement of benzodiazepine binding by the novel anxiolytic ICI 136,753. *Soc. Neurosci.* 6:190 (*Abstr.*)
169. Supavilai, P., Karobath, M. 1979. Stimulation of benzodiazepine receptor binding by SQ 20009 is chloride-

dependent and picrotoxin-sensitive. *Eur. J. Pharmacol.* 60:111–13

170. Supavilai, P., Karobath, M. 1981. Action of pyrazolpyridines as modulators of (^{3}H) flunitrazepam binding to the GABA/benzodiazepine receptor complex of the cerebellum. *Eur. J. Pharmacol.* 70:183–93
171. Leeb-Lundberg, F., Snowman, A., Olsen, R. W. 1980. Barbiturate receptors are coupled to benzodiazepine receptors. *Proc. Natl. Acad. Sci. USA* 77:7468–72
172. Skolnick, P., Moncada, V., Barker, J. L., Paul, S. M. 1981. Pentobarbital: dual actions to increase brain benzodiazepine receptor affinity. *Science* 211:1448–50
173. Ticku, M. K. 1981. Interaction of depressant, convulsant and anticonvulsant barbiturates with [^{3}H]-diazepam binding site at the benzodiazepine-GABA-receptor-ionophore complex. *Biochem. Pharmacol.* 30:1573–79
174. Davis, W. C., Ticku, M. K. 1981. Pentobarbital enhances [^{3}H] diazepam binding to soluble receptors at the benzodiazepine-GABA-receptor-ionophore complex. *Neurosci. Lett.* 23:209–13
175. Leeb-Lundberg, F., Olsen, R. W. 1982. Interactions of barbiturates of various pharmacological categories with benzodiazepine receptors. *Mol. Pharmacol.* In press
176. Olsen, R. W., Leeb-Lundberg, F. 1981. Convulsant and anticonvulsant drug binding sites related to the GABA receptor/ionophore system. In *Neurotransmitters, Seizures and Epilepsy,* ed. P. L. Morselli, K. G. Lloyd, W. Löscher, B. S. Meldrum, E. H. Reynolds, pp. 151–63. New York: Raven
177. Butler, T. C. 1942. The delay in onset of action of intravenously injected anaesthetics. *J. Pharmacol.* 74:118–28
178. Huang, L.-Y., Barker, J. L. 1980. Pentobarbital: stereospecific actions of the (+) and (–) isomers revealed on cultured mammalian neurons. *Science* 207:195–97

178a. Williams, M., Yarbrough, G. G. 1979. Enhancement of in vitro binding and some of the pharmacological properties of diazepam by a novel anthelmintic agent, avermectin B_{1a}. *Eur. J. Pharmacol.* 56:273–76

179. Fritz, L. C., Wang, C. C., Gorio, A. 1979. Avermectin B_{1a} irreversibly blocks postsynaptic potentials at the lobster neuromuscular junction by reducing muscle membrane resistance. *Proc. Natl. Acad. Sci. USA* 76:2062–66
180. Paul, S. M., Skolnick, P., Zatz, M. 1980. Avermectin B_{1a}: an irreversible activator of the γ-aminobutyric acid-benzodiazepine-chloride ionophore receptor complex. *Biochem. Biophys. Res. Commun.* 96:632–38
181. Supavilai, P., Karobath, M. 1981. *In vitro* modulation by Avermectin B_{1a} of the GABA/benzodiazepine receptor complex of rat cerebellum. *J. Neurochem.* 36:798–803
182. Pong, S. S., Wang, C. C. 1980. The specificity of high affinity binding of Avermectin B_{1a} to mammalian brain. *Neuropharmacology* 19:311–17
183. Skolnick, P., Lock, K.-L., Paul, S. M., Marangos, P. J., Jones, R., Irmscher, K. 1980. Increased benzodiazepine receptor number elicited in vitro by a novel purine, EMD 28422. *Eur. J. Pharmacol.* 67:179–86
184. Burch, T. P., Ticku, M. K. 1980. Ethanol enhances [^{3}H] diazepam binding at the benzodiazepine-GABA receptor-ionophore complex. *Eur. J. Pharmacol.* 67:325–26
185. Davis, W. C., Ticku, M. K. 1981. Solubilization of the picrotoxinin binding receptor from mammalian brain. *J. Neurochem.* 36:1572–79
186. Tunnicliff, G., Smith, J. A. 1979. Competition for diazepam receptor binding by diphenylhydantoin and its enhancement by γ-aminobutyric acid. *Biochem. Biophys. Res. Commun.* 91:1018–24
187. Gallager, D. W., Mallorga, P., Tallman, J. F. 1980. Interaction of diphenylhydantoin and benzodiazepines in the CNS. *Brain Res.* 189:209–20

187a. Paul, S., Skolnick, P. 1978. Rapid changes in brain benzodiazepine receptors after experimental seizures. *Science* 202:892–94

188. McNamara, J. O., Peper, A. M., Patrone, V. 1980. Repeated seizures induce long-term increase in hippocampal benzodiazepine receptors. *Proc Natl. Acad. Sci. USA* 77:3029–32
189. Speth, R. C., Bresolin, N., Yamamura, H. I. 1979. Acute diazepam administration produces rapid increases in brain benzodiazepine receptor density. *Eur. J. Pharmacol.* 59:159–60
190. Rosenberg, H. C., Chiu, T. H. 1981. Tolerance during chronic benzodiazepine treatment associated with decreased receptor binding. *Eur. J. Pharmacol.* 70:453–60
191. Möhler, H., Okada, T., Enna, S. J. 1978. Benzodiazepine and neurotransmitter receptor binding in rat brain after

chronic administration of diazepam or phenobarbital. *Brain Res.* 156:391–95

192. Braestrup, C., Nielsen, M., Squires, R. F. 1979. No changes in rat benzodiazepine receptors after withdrawal from continuous treatment with lorazepam and diazepam. *Life Sci.* 24:347–51
193. Sonawane, B. R., Yaffe, S. J., Shapiro, B. H. 1980. Changes in mouse brain diazepam receptor binding after phenobarbital administration. *Life Sci.* 27: 1335–38
194. Leeb-Lundberg, F., Olsen, R. W. 1981. Brain regional variation in benzodiazepine and GABA receptor association. *Soc. Neurosci.* 7:738 (Abstr.)
195. O'Brien, R. A., Spirt, N. M. 1980. The inhibition of GABA-stimulated benzodiazepine binding by a convulsant benzodiazepine. *Life Sci.* 26:1441–45
196. Karobath, M., Drexler, G., Supavilai, P. 1981. Modulation by picrotoxin and IPTBO of ^{3}H-flunitrazepam binding to the GABA/benzodiazepine receptor complex of rat cerebellum. *Life Sci.* 28:307–13
197. Fujimoto, M., Okabayashi, T. 1981. Effect of picrotoxin on benzodiazepine receptors and GABA receptors with reference to the effect of Cl^- ion. *Life Sci.* 28:895–901
198. Skolnick, P., Paul, S. M., Barker, J. L. 1980. Pentobarbital potentiates GABA-enhanced [^{3}H] diazepam binding to benzodiazepine receptors. *Eur. J. Pharmacol.* 65:125–27
199. Placheta, P., Karobath, M. 1980. In vitro modulation by SQ 20009 and SQ 65396 of GABA receptor binding in rat CNS membranes. *Eur. J. Pharmacol.* 62:225–28
200. Willow, M. 1981. A comparison of the action of pentobarbitone and etomidate on [^{3}H]-GABA binding to crude synaptosomal rat brain membranes. *Brain Res.* 220:427–31
201. Willow, M., Johnston, G. A. R. 1980. Enhancement of GABA binding by pentobarbitone. *Neurosci. Lett.* 18: 323–27
202. Willow, M., Johnston, G. A. R. 1981. Dual action of pentobarbitone on GABA binding: role of binding site integrity. *J. Neurochem.* 37:1291–94
203. Willow, M., Johnston, G. A. R. 1981. Enhancement by anesthetic and convulsant barbiturates of GABA binding to rat brain synaptosomal membranes. *J. Neurosci.* 1:364–67
204. Willow, M., Johnston, G. A. R. 1981. Pentobarbitone slows the dissociation of GABA from rat brain synaptosomal binding sites. *Neurosci. Lett.* 23:71–74
205. Olsen, R. W., Snowman, A., Leeb-Lundberg, F. 1981. Barbiturates enhance GABA receptor binding in mammalian brain membranes *in vitro*. *Fed. Proc.* 40:309
206. DeFeudis, F. V., Ossola, L., Mandel, P. 1979. More muscimol binding sites than GABA binding sites in a particulate fraction of rat brain. *Biochem. Pharmacol.* 28:2687–89
207. Hill, D. R., Bowery, N. G. 1981. ^{3}H-baclofen and ^{3}H-GABA bind to bicuculline-insensitive $GABA_B$ sites in rat brain. *Nature* 290:149–52
208. Study, R. E., Barker, J. L. 1981. Diazepam and (–) pentobarbital: fluctuation analysis reveals different mechanisms for potentiation of GABA responses in cultured central neurons. *Proc. Natl. Acad. Sci. USA.* 78:7180–84
209. Birdsall, N. J. M., Berrie, C. P., Burgen, A. S. V., Hulme, E. C. 1980. Modulation of the binding properties of muscarinic receptors: evidence for receptor-effector coupling. *Adv. Biochem. Psychopharmacol.* 20:107–16