Proc. Natl. Acad. Sci. USA
Vol. 77, No. 12, pp. 7468–7472, December 1980
Neurobiology

Barbiturate receptor sites are coupled to benzodiazepine receptors

(anesthetic/anticonvulsant/γ-aminobutyric acid receptor/Cl^- channel)

FREDRIK LEEB-LUNDBERG, ADELE SNOWMAN, AND RICHARD W. OLSEN

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

Communicated by George A. Zentmyer, August 11, 1980

ABSTRACT **Barbiturates enhance the binding of [^{3}H]diazepam to benzodiazepine receptor sites in rat brain. This effect occurs at pharmacologically relevant concentrations of barbiturates, and the relative activity of a series of compounds correlates highly with anesthetic activity of the barbiturates and with their ability to enhance postsynaptic inhibitory responses to the neurotransmitter γ-aminobutyric acid. Barbiturate enhancement of benzodiazepine binding is stereospecific, with the more active anesthetic isomers of N^1-methylbarbiturates being also more active than their stereoisomers in enhancing benzodiazepine binding. The active barbiturates produce a reversible enhancement in the affinity of specific benzodiazepine binding with no effect on the number of binding sites. The barbiturate enhancement, but not the baseline benzodiazepine binding, is competitively inhibited by the convulsant picrotoxinin (at 1–10 μM), a drug that has been shown to label barbiturate-sensitive brain membrane sites related to the γ-aminobutyric acid receptor–ionophore complex. The barbiturate effect is also dependent upon the presence of certain anions, and only those anions, that penetrate the chloride channels regulated by γ-aminobutyric acid receptors. These results suggest that picrotoxin-sensitive barbiturate binding sites are coupled to benzodiazepine receptors in the γ-aminobutyric acid receptor–ionophore complex, and that these binding sites have the properties of pharmacologically relevant receptors that mediate at least part of the action of various nervous system depressant and excitatory drugs.**

Barbiturates are general central nervous system depressants that are used clinically for anesthetic, sedative-hypnotic, and anticonvulsant actions. The site (or sites) of action of these drugs is generally considered to be the excitable nerve membrane, perhaps involving a decrease in excitatory synaptic transmission (1, 2) or an increase in inhibitory synaptic transmission (2, 3). It is not known whether the drug action involves any specific receptor sites or more nonspecific membrane perturbations. The latter type of effects are known to occur in biological systems exposed to high concentrations (≥0.3 mM) of barbiturates (4), whereas anesthetic actions are thought to involve somewhat lower concentrations (1–200 μM) (1).

Recent electrophysiological studies suggest that barbiturates can enhance or mimic the postsynaptic response to the inhibitory neurotransmitter γ-aminobutyric acid (GABA) (2, 3, 5, 6). This appears to involve a potentiation of chloride ion conductance activated by GABA binding to its receptor site (3). Barbiturates can directly activate chloride ion channels or prolong the lifetime of GABA-activated channels (3), an action which will also effectively reverse the antagonism of GABA postsynaptic responses by bicuculline (7) and picrotoxin (2). *In vitro* studies suggest that at therapeutic concentrations, barbiturates do not affect GABA receptor binding or presynaptic synthesis, release, or reuptake of GABA (8). A possible site of action involves central nervous system membrane sites labeled with radioactive picrotoxinin (α-[^{3}H]dihydropicrotoxinin, [^{3}H]DHP) (9, 10). [^{3}H]DHP binding is inhibited by micromolar concentrations of depressant barbiturates (9, 10), submicromolar concentrations of excitatory barbiturates (9, 10), and other convulsant drugs that can block GABA synapses (10, 11).

Another class of central nervous system-depressant drugs that may act at least in part by enhancement of GABAergic inhibitory synaptic transmission (5) are the benzodiazepines (BZ), such as diazepam. These compounds bind to high-affinity receptor sites in the central nervous system with a chemical specificity that correlates well with the anxiolytic, anticonvulsant, hypnotic, and muscle-relaxant activity of BZ (12, 13). The BZ receptors are at least partly coupled to GABA receptor binding sites in the central nervous system, as shown by allosteric activation by GABA of BZ binding in membranes (14). Consistent with the different pharmacological profiles of barbiturates and BZ, barbiturates do not inhibit BZ binding sites (12–14), and no *in vitro* coupling has so far been demonstrated between barbiturates or other drugs binding to the picrotoxinin-labeled sites and GABA receptors (8–11).

We now provide evidence for *in vitro* coupling between barbiturate/picrotoxin binding sites and BZ receptors in the central nervous system. This barbiturate binding site has a specificity that correlates very well with the anesthetic and GABA-enhancing actions of these drugs and, thus, can be designated as a barbiturate receptor.

MATERIALS AND METHODS

[*N-methyl*-^{3}H]Diazepam (83.5 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) was purchased from New England Nuclear. Nonradioactive diazepam was donated by H. Möhler (Hoffmann–LaRoche). (+)- and (−)-hexobarbital and (+)- and (−)-N^1-methyl-5-phenyl-5-propylbarbituric acid (MPPB) were gifts of J. Knabe, University of Saarlandes. (+)- and (−)-pentobarbital were gifts of J. Barker, National Institutes of Health. 1,3-Dimethylbutylbarbituric acid (DMBB), amobarbital, and secobarbital were gifts of Eli Lilly. Barbital was a gift of Merck. Mephobarbital and phenobarbital were gifts of Sterling–Winthrop. All other drugs and materials were obtained from commercial sources.

White Sprague–Dawley rats (200–300 g) were decapitated, and their brains were rapidly removed and bathed in ice-cold 0.32 M sucrose. Cerebral cortex was then removed and homogenized in 20 vol of 0.32 M sucrose in a glass homogenizer fitted with a Teflon pestle (12 passes, 400 rpm). The homogenate was centrifuged at 2000 rpm (1000 × *g*) for 10 min at 0–4°C in a Beckman JA17 rotor. The supernatant fraction was saved and the pellet (P_1) was discarded. The supernatant

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: GABA, γ-aminobutyric acid; [^{3}H]DHP, α-[^{3}H]dihydropicrotoxinin; BZ, benzodiazepine(s); DMBB, 1,3-dimethylbutylbarbituric acid; CHEB, 5-ethyl-5-(2-cyclohexylideneethyl)barbituric acid; MPPB, N^1-methyl-5-phenyl-5-propylbarbituric acid.

fraction was centrifuged at 45,000 rpm (140,000 × *g*) for 45 min in a Spinco rotor 60 Ti. The pellet ($P_2 + P_3$) was resuspended [Ultra-turrax (Cincinnati, OH), 5 sec at setting 40%] in 25 vol of ice-cold double-distilled water (osmotic shock) and centrifuged at 45,000 rpm for 45 min. The $P_2 + P_3$ pellet was washed again in buffer (0.2 M NaCl/20 mM sodium phosphate, pH 7.0 ± 0.1). This procedure is intended to lower membrane GABA levels below the micromolar concentrations that affect BZ binding (14) while minimizing damage to the fragile binding sites for picrotoxinin and barbiturates (8–11). The pellet was resuspended to a final protein concentration of 0.5–2.0 mg/ml in buffer.

Aliquots (0.8 ml) of the membrane suspension were incubated in triplicate for 60 min at 0°C with 0.5 nM [^{3}H]diazepam with and without drugs and in a total volume of 1 ml. At the end of the incubation, the membranes were rapidly trapped on Whatman GF/B filters. Two 2-ml additions of 0.2 M NaCl were added to the incubation vial and poured onto the filter. The filters were dried and put into plastic vials. Five milliliters of Aquasol/toluene, 2:1 (vol/vol; New England Nuclear), were added, and vials were assayed for radioactivity in a Beckman 3155T scintillation counter. Efficiency (35%) was routinely determined with [^{3}H]toluene. Background was determined in the presence of 10 μM unlabeled diazepam and usually was 5% of the total radioactivity.

RESULTS

Anesthetic barbiturate compounds enhanced the binding of [^{3}H]diazepam to rat brain homogenates. The increase in BZ binding in the presence of barbiturates was due to a change in binding affinity, with no change in the number of BZ binding sites detected. Fig. 1 shows a Scatchard plot for [^{3}H]diazepam binding to washed crude membrane fractions from rat cerebral cortex, giving (*i*) a K_d of 2.23 nM and a B_{max} of 0.99 pmol/mg of protein in the absence of added drugs and (*ii*) a K_d of 0.86 nM and a B_{max} of 0.99 pmol/mg of protein in the presence of pentobarbital at 500 μM, a concentration that gives the maximal effect (Fig. 2). This enhancement of BZ binding was fully reversible in that if membranes treated with barbiturate were centrifuged (30 min at 200,000 × *g*), the resuspended pellets demonstrated BZ binding equivalent to the untreated controls (data not shown).

The enhancement of BZ binding by barbiturates was concentration dependent (Figs. 2, 3, and 4), with noticeable effects at 10 μM and saturation at about 1 mM. The enhancement of BZ binding by barbiturates was inhibited by picrotoxinin in a concentration-dependent manner, with 50% reversal of pentobarbital enhancement of BZ binding obtained with 3 μM picrotoxinin. Fig. 2 shows a double reciprocal plot of the enhancement of BZ binding by varying concentrations of pentobarbital with and without 100 μM picrotoxinin. The linearity of the control plot is consistent with a concentration dependence of pentobarbital enhancement that follows a Michaelis–Menten-type saturation equation with an apparent single K_m (concentration giving half-maximal effect, or EC_{50}) of 150 μM. The inhibition of the pentobarbital enhancement of BZ binding by picrotoxinin is competitive (increases the apparent EC_{50} for pentobarbital but does not affect the maximal effect at the saturating concentration of pentobarbital), with an apparent K_i of 10 μM.

Barbiturate enhancement of BZ binding showed a marked dependence on the presence of certain anions. Fig. 3 shows that the apparent EC_{50} value was lowered and the maximal effect was enhanced by increasing the concentration of chloride ions. The slope of the barbiturate concentration-dependence curve also varied with different concentrations of the anion. The data

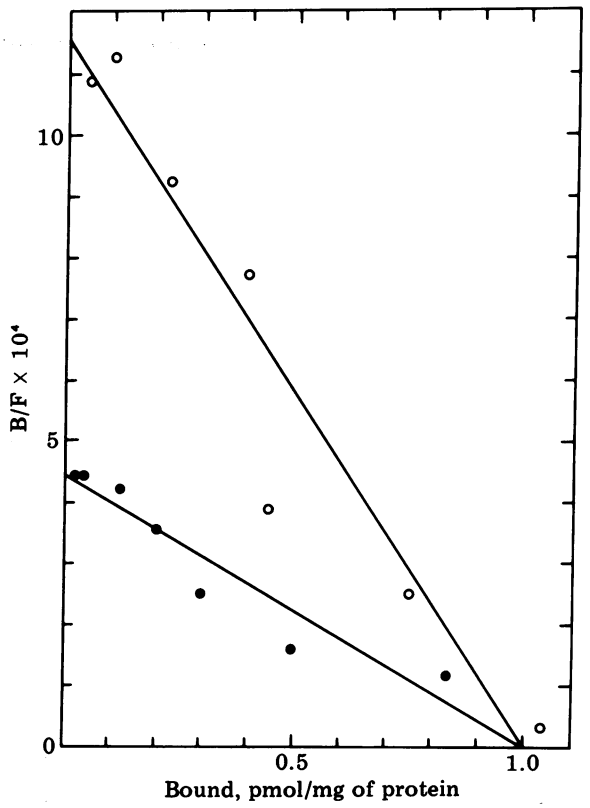

FIG. 1. Scatchard plot of [^{3}H]diazepam binding to rat cortex in the absence and presence of barbiturate. [^{3}H]Diazepam concentrations were varied from 0.05 nM to 50 nM, and nondisplaceable background was determined with 10 μM nonradioactive diazepam. ●, Control; ○, with (±)-pentobarbital (500 μM). The solid lines represent computer-fitted linear regressions. The result is typical of five experiments; an effect on the K_d of diazepam binding was also observed with 100 μM pentobarbital or 100 μM secobarbital.

in Fig. 3 with barbiturate are in each case compared to control ^{3}H-labeled BZ binding carried out in buffer containing the corresponding identical chloride concentration without added barbiturate. At a given concentration of barbiturate, approximately 20–30 mM chloride was needed for a half-maximal effect and at least 100 mM chloride for the maximal effect.

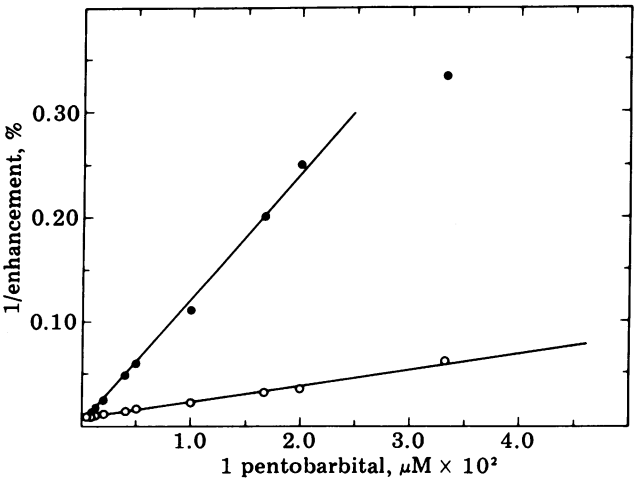

FIG. 2. Competitive inhibition by picrotoxinin of pentobarbital enhancement of BZ binding: double reciprocal plot. Rat cortex membranes were prepared and assayed for [^{3}H]diazepam binding (at 0.5 nM). Varying concentrations of (±)-pentobarbital (1 μM to 1 mM) were included in the assays without (○) or with (●) 100 μM picrotoxinin. The result is typical of three experiments.

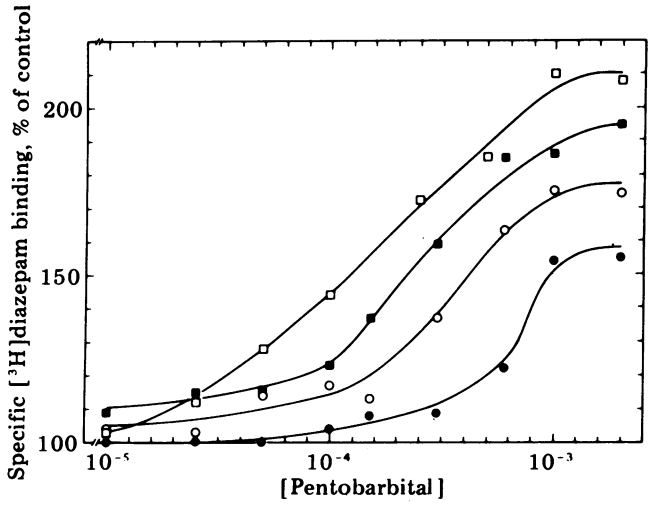

FIG. 3. Concentration dependence of pentobarbital enhancement of BZ binding at various concentrations of NaCl. All assays included 10 mM NaH_2PO_4/Na_2HPO_4 (pH 7.0). ●, 18 mM NaCl; ○, 35 mM NaCl; ■, 70 mM NaCl; □, 200 mM NaCl. The points represent the average of two experiments in triplicate, which varied by <10%.

This ion requirement did not involve a nonspecific ionic strength effect, because only some anions (Table 1) could support the barbiturate enhancement of BZ binding. Nor did it involve the cation, because several metal chloride salts were equally effective, and both sodium and potassium salts of an inactive anion such as acetate were equally ineffective. Interestingly, the only anions that were effective in this regard (Table 1) were those anions shown by others (15) to permeate GABA-regulated anion channels involved in inhibitory synapses in the spinal cord. The same anion specificity was observed for basal (16) and etazolate-enhanced (17) BZ binding.

The ability of a series of barbiturate compounds to enhance [³H]diazepam binding (Fig. 4; Table 2) agreed very well with their relative potencies as anesthetics or in the effective reversal of GABA synaptic antagonists. Fig. 5 shows the correlation

Table 1. Effect of anions on barbiturate enhancement of BZ binding*

Ion	% of baseline†
KI	140
KBr	136
NaBr	149
NaCl	134
KCl	141
$CaCl_2$	152
$NaNO_3$	133
NaSCN	135
NaOOCH	125

* Membranes were prepared and assayed for [³H]diazepam binding as described in *Materials and Methods*.

† Specific binding of [³H]diazepam in the presence of 100 μM pentobarbital divided by the binding its absence, ×100. Assays contained 10 mM Na_2HPO_4/NaH_2PO_4, pH 7.0, and the salt indicated at 100 mM. The following salts gave no significant (≤6%) enhancement by pentobarbital of BZ binding: NaN_3, Na_2SO_4, Na_2SO_3, $NaOOCCH_3$, $KOOCCH_3$, $NaHCO_3$, NaF, $NaOOCCH_2CH_3$, $KOOCCH_2CH_3$, and Na_2HPO_4/NaH_2PO_4. All of the anions listed in the table can substitute for Cl^- in electrophysiological measurement of inhibitory synaptic potentials, whereas none of the inactive anions listed will substitute (15). [N_3^- is a possible exception, but its effect on neurons may be indirect (15)]. Each result is the average of at least two determinations, which varied by <15%.

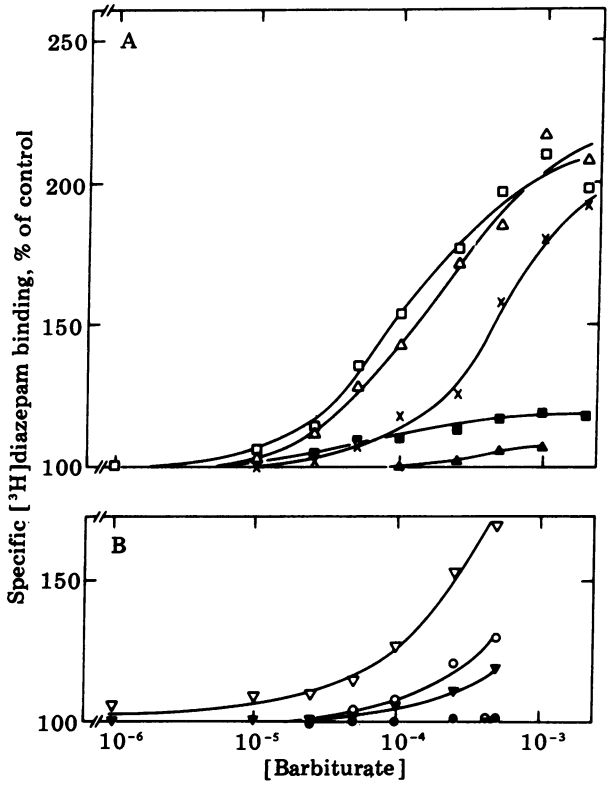

FIG. 4. Concentration dependence for various barbiturates in enhancement of BZ binding. Membranes were prepared and assayed for [³H]diazepam binding. □, secobarbital; △, pentobarbital; x, amobarbital; ■, mephobarbital; ▲, phenobarbital; ▽, (+)-hexobarbital; ▼, (−)-hexobarbital; ○, (−)-MPPB; (●), (+)-MPPB; ◒, (+)-MPPB and (−)-MPPB. The results are the average of two experiments, which varied by <10%.

obtained for comparison of the barbiturate enhancement of BZ binding with the barbiturate reversal of bicuculline antagonism of GABA responses in the isolated sympathetic ganglion (7) and

Table 2. Relative activity of barbiturates in enhancing BZ binding*

Compound	% enhancement†	Relative activity‡
(±)-DMBB	119 ± 10	1.40
Secobarbital	97 ± 10	1.14
(−)-Pentobarbital	87 ± 8	1.02
(±)-Pentobarbital	85 ± 8	1.00
(+)-Hexobarbital	69 ± 7	0.81
(+)-Pentobarbital	65 ± 6	0.76
Amobarbital	58 ± 6	0.68
(−)-MPPB	30 ± 7	0.35
(−)-Hexobarbital	19 ± 5	0.22
CHEB	15 ± 5	0.18
Phenobarbital	6 ± 6	0.07
Metharbital	3 ± 3	0.035
Barbital	3 ± 3	0.035
(+)-MPPB	2 ± 2	0.024
(+)-MPPB and (−)-MPPB	2 ± 2	0.024

* Rat brain membranes were prepared and assayed for [³H]diazepam binding.

† Percentage enhancement refers to the increase in [³H]diazepam specifically bound in the presence of 500 μM barbiturate compared to that bound in the absence of barbiturate, divided by this control level of binding, ×100. Each result is the average of three experiments ± SEM.

‡ Relative activities for % enhancement of benzodiazepine binding compared to (±)-pentobarbital as 1.00.

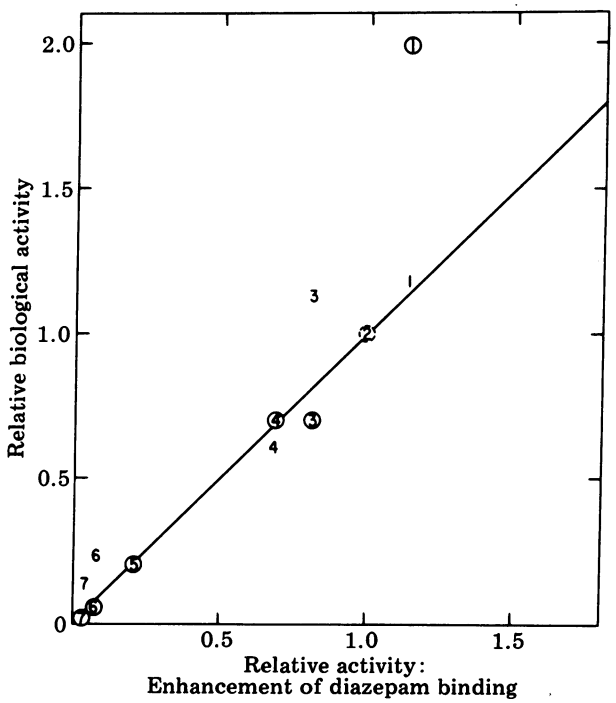

FIG. 5. Correlation between barbiturate biological activity and enhancement of BZ binding. All barbiturates are compared to a relative activity of 1.0 for pentobarbital, as shown in Table 2 for enhancement of BZ binding. Relative biological activities for reversal of GABA antagonists (circled numbers) or anesthetic potency (uncircled numbers) are taken from ref. 7. —, Theoretical slope of 1.0; 1, secobarbital; 2, pentobarbital; 3, (±)-hexobarbital; 4, amobarbital; 5, mephobarbital; 6, phenobarbital; 7, barbital.

also with barbiturate-induced anesthesia in the intact animal (18). A comparison of seven points (circled numbers) with the former data yields a correlation coefficient of 0.913 ($P < 0.005$) or 0.994 ($P < 0.001$) if point no. 1 is omitted, and a comparison of six points with the latter data (uncircled numbers) yields a correlation coefficient of 0.956 ($P < 0.005$). The experimental points also fall near the theoretical line having a slope of one.

Stereospecificity was also observed for barbiturate enhancement of BZ binding. The active hypnotic (+)-hexobarbital was much more effective than the less active hypnotic (−)-hexobarbital (19). Likewise, the depressant (−) isomer of MPPB enhanced BZ binding more potently than the (+) isomer, which has been reported to produce central nervous system excitation rather than depression (20). The presence of (+)-MPPB could also reverse the enhancement of BZ binding by (−)-MPPB (Fig. 4*B*; Table 2). The (−) isomer of pentobarbital was more potent than the (+) isomer; the former compound is reported to depress neuronal activity, whereas the latter has some excitatory action on cells (3).

Another convulsant barbiturate, 5-ethyl-5-(2-cyclohexylideneethyl)barbituric acid (CHEB) gave only weak enhancement of BZ binding. However, DMBB was the most potent of all barbiturates tested in enhancing BZ binding (Table 2). This racemic mixture contains both the excitatory (+) isomer and the depressant (−) isomer; the (−) isomer has been reported to have hypnotic activity greater than that of pentobarbital (21). Thus, the enhancement of BZ binding by the (±) pair could be due to the (−) isomer.

The barbiturate enhancement of benzodiazepine binding to sites that are known to be coupled to postsynaptic GABA receptor–ionophores, the chemical specificity of the effect, its reversal by picrotoxinin, and the anion requirement all support the conclusion that this *in vitro* barbiturate action involves a pharmacologically relevant receptor site for these drugs.

DISCUSSION

Although picrotoxin binding sites in mammalian brain membranes have been shown to be inhibited specifically by drugs that affect the postsynaptic response to the neurotransmitter GABA (8–11), no *in vitro* interactions between [^{3}H]DHP binding sites and GABA receptor binding sites have so far been reported. On the other hand, the binding of GABA agonists to GABA receptor sites causes an enhancement in the *in vitro* binding of the BZ to pharmacologically relevant receptor sites (14), which suggests a physical coupling between these two classes of receptors, at least in part.

Another class of drugs, the pyrazolopyridine compounds (e.g., etazolate, or SQ 20009), enhances BZ binding *in vitro*, but not by a direct interaction of this drug with GABA receptors (22, 23). The enhancement of BZ binding by etazolate was found to be inhibited by picrotoxin (17). We have shown that enhancement of BZ binding by etazolate is reversed by picrotoxinin and other convulsants at similar concentrations to those that inhibit [^{3}H]DHP binding (24, 25), indicating that etazolate acts via the picrotoxin receptor. It follows logically that other depressant drugs, such as barbiturates, that bind to [^{3}H]DHP binding sites, might also perturb BZ binding *in vitro*, and we present evidence here that barbiturates enhance the binding of [^{3}H]diazepam to rat brain membranes.

This enhancement of BZ binding by barbiturates, as in the case of GABA (14) or etazolate (17, 22, 23) enhancement of BZ binding, involves an increased affinity for BZ rather than any change in the number of binding sites. In the presence of saturating GABA concentrations (and the maximal GABA enhancement of BZ binding), barbiturates or etazolate will further enhance BZ binding in an additive fashion, whereas the effects of maximal etazolate and barbiturates are not additive (unpublished data). This is consistent with etazolate and barbiturates acting at the same site (a picrotoxin-sensitive site), which is distinct from the GABA receptor site. Furthermore, the enhancement of BZ binding by GABA is not strictly anion dependent (17, 22, 23), whereas the enhancement by both etazolate (17) and barbiturates (this study) is strictly dependent upon certain anions. Also, GABA enhancement is blocked by 20 μM piperidine-4-sulphonic acid and imidazole acetic acid (14), whereas that of barbiturates or etazolate is not (unpublished data). This latter observation also shows that the barbiturate effect is not mediated by GABA.

The anions that will support barbiturate enhancement of BZ binding are the same anions that enhance basal BZ binding (16) and are exactly those anions that permeate anion channels activated in spinal cord inhibitory synapses (15), now known to be GABAergic (2). Similar concentrations (20–30 mM) of chloride ions are required for half-maximal enhancement of baseline (16) or the barbiturate enhancement (this study) of BZ binding. A similar ion specificity was observed for enhancement of bicuculline inhibition of GABA receptor binding, whereas the apparent affinity of a GABA agonist such as 3-aminopropanesulfonic acid was not altered (26). These results are consistent with the suggestion that both BZ receptors (16) and the barbiturate receptor sites described here are at least partly coupled to chloride channels and that these receptors mediate the action of these drugs in enhancing the function of GABA receptor-activated chloride channels.

Drugs that bind to picrotoxin sites, such as barbiturates (9–11), etazolate (24, 25; unpublished data), and a convulsant BZ, RO5-3663 (10, 24, 25), have been shown to produce both modest allosteric perturbations of GABA receptor binding *in*

vitro (27, 28) and modulation of GABA enhancement of BZ binding (28–30). In agreement with our results, barbiturates were also seen to enhance baseline BZ binding (30). In the light of all these *in vitro* interactions that take place in cell-free membranes at 0°C on a very rapid time scale and corresponding interactions of barbiturates with GABA-regulated chloride channels in intact cells (3), it seems likely that the membrane macromolecules must be intimately associated in a membrane complex. Accordingly, we have proposed a three receptor/chloride ion channel model for the postsynaptic GABA receptor–ionophore complex (24, 25).

The activity for several barbiturates in enhancing BZ binding *in vitro* correlates well with pharmacological activity of these drugs measured both in an electrophysiological study with isolated central nervous system tissue (7) and in the intact animal (18), despite obvious potential pharmacokinetic problems in the latter case (i.e., drug metabolism, tissue distribution, serum protein binding, etc. can alter the *in vivo* drug potencies). This correlation strongly suggests that the barbiturate receptor that is coupled to the BZ receptor is involved in the central nervous system depressant actions of barbiturates. No such correlation can be made with the antiepileptic action of barbiturates, but it is possible that this drug receptor plays some role in the anticonvulsant action of barbiturates as well.

The barbiturate enhancement of BZ binding was stereospecific, as required for the stereospecific anesthetic activity of N^1-methyl barbiturates, such as hexobarbital and MPPB: The (+)-hexobarbital and the (−)-MPPB isomers are more potent than their stereoisomers in both anesthetic potency (19, 20) and in this binding assay.

Whereas convulsant barbiturates are more potent than depressant barbiturates in inhibiting DHP binding (9, 11), some convulsant barbiturates such as CHEB only weakly enhanced BZ binding and did not inhibit the enhancement by other barbiturates. (±)-DMBB, on the other hand, potently enhanced BZ binding. This barbiturate contains an optically active carbon in one of the C^5 sidechains (1,3-dimethylbutyl), and the (+) isomer is excitatory whereas the (−) isomer is depressant (21). We have used the racemic pair in these studies, the pure isomers being unavailable. Nevertheless, the lack of a potent picrotoxin-like effect of (±)-DMBB and CHEB on the barbiturate enhancement of BZ binding is unexplained. Another barbiturate with an optically active carbon in the C^5 side chain is pentobarbital. Here the (−) isomer is more active in the enhancement of BZ binding. This isomer produces an inhibitory chloride conductance in neurons and a smooth hypnosis in intact animals, whereas the (+) isomer can excite neurons and induce excitability in animals before they become anesthetized by the drug (3).

It might be only the long-term depressant action of barbiturates that is related to enhancement of BZ binding, or perhaps the sites for both depressant and excitatory actions of barbiturates are coupled to BZ receptors, and the relative allosteric perturbation is unrelated to mechanism in intact cells. Another possible interpretation involves multiple populations of [^{3}H]-DHP binding sites. Some convulsant drugs, notably (±)-DMBB and CHEB (9, 11), gave shallow concentration-dependence curves for the displacement of [^{3}H]DHP binding, suggesting a possible heterogeneity of binding sites for these drugs. Although the enhancement of BZ binding by barbiturates is blocked by concentrations of picrotoxinin (1–10 μM) similar to those binding to [^{3}H]DHP sites, and although the barbiturates are effective at concentrations similar to those inhibiting DHP binding, there is not a perfect correlation between IC_{50} values for [^{3}H]DHP binding and EC_{50} values for enhancement of BZ binding by barbiturates [e.g., (±)-DMBB is not 100–1000 times more potent than pentobarbital is, as it is for [^{3}H]DHP binding, and CHEB and mephobarbital are weaker than pentobarbital in enhancing BZ binding but are more potent in inhibiting [^{3}H]DHP binding]. This suggests that the pharmacologically important barbiturate receptor site described here, which is picrotoxin-sensitive, is either a subpopulation of sites labeled by [^{3}H]DHP or another receptor site with very similar properties.

This research was supported by National Science Foundation Grant BNS 77-24414; National Institutes of Health Grants NS12422, RR09070, and AM 07310; and Research Career Development Award NS 00224 to R.W.O.

1. Richards, C. D. (1972) *J. Physiol. (London)* **227,** 749–767.
2. Nicoll, R. A. & Wojtowicz, J. M. (1980) *Brain Res.* **191,** 225–237.
3. Huang, L.-Y. M. & Barker, J. L. (1980) *Science* **207,** 195–197.
4. Seeman, P. (1972) *Pharmacol. Rev.* **24,** 583–655.
5. Haefely, W., Polc, P., Schaffner, R., Keller, H. H., Pieri, L. & Möhler, H. (1979) in *GABA-Neurotransmitters,* eds. Krogsgaard-Larsen, P., Scheel-Kruger, J. & Kofod, H. (Munksgaard, Copenhagen), pp. 357–375.
6. Evans, R. H. (1979) *Brain Res.* **171,** 113–120.
7. Bowery, N. G. & Dray, A. (1978) *Br. J. Pharmcol.* **63,** 197–215.
8. Olsen, R. W., Ticku, M. K., Van Ness, P. C. & Greenlee, D. V. (1978) *Brain Res.* **139,** 277–294.
9. Ticku, M. & Olsen, R. W. (1978) *Life Sci.* **22,** 1643–1652.
10. Olsen, R. W., Leeb-Lundberg, F. & Napias, C. (1980) *Brain Res. Bull.* 5, suppl. 2, 217–221.
11. Olsen, R. W., Ticku, M. K., Greenlee, D. & Van Ness, P. (1979) in *GABA-Neurotransmitters,* eds. Krogsgaard-Larsen, P., Scheel-Kruger, J. & Kofod, H. (Munksgaard, Copenhagen), pp. 165–178.
12. Squires, R. F., & Braestrup, C. (1977) *Nature (London)* **266,** 732–734.
13. Möhler, H. & Okada, T. (1977) *Science* **198,** 849–851.
14. Tallman, J. F., Paul, S. M., Skolnick, P. & Gallager, D. W. (1980) *Science* **207,** 274–281.
15. Araki, T., Ito, M. & Oscarsson, O. (1961) *J. Physiol. (London)* **159,** 410–435.
16. Costa, T., Rodbard, D. & Pert, C. B. (1979) *Nature (London)* **277,** 315–317.
17. Supavilai, P. & Karobath, M. (1979) *Eur. J. Pharmacol.* **60,** 111–113.
18. Butler, T. C. (1942) *J. Pharmacol.* **74,** 118–128.
19. Wahlstrom, G., Buch, H. & Buzello, W. (1970) *Acta Pharmacol. Toxicol.* **28,** 493–498.
20. Grossmann, W., Jurna, J. & Theres, C. (1974) *Naunyn-Schmiedeberg's Arch. Pharmakol.* **282,** 367–377.
21. Downes, H., Perry, R. S., Ostlund, R. E. & Karler, R. (1970) *J. Pharmacol. Exp. Ther.* **175,** 692–699.
22. Williams, M. & Risley, E. A. (1979) *Life Sci.* **24,** 833–842.
23. Beer, B., Klepner, C. A., Lippa, A. S. & Squires, R. F. (1978) *Pharmacol. Biochem. Behav.* **9,** 849–851.
24. Leeb-Lundberg, F. & Olsen, R. W. (1981) in *Psychopharmacology and Biochemistry of Neurotransmitter Receptors,* eds. Yamamura, H. I., Olsen, R. W. & Usdin, E. (Elsevier, New York), *in press.*
25. Olsen, R. W. & Leeb-Lundberg, F. (1981) in *GABA and Benzodiazepine Receptors,* eds. Costa, E., DiChiara, G. & Gessa, G. (Raven, New York), pp. 93–102.
26. Enna, S. J. & Snyder, S. H. (1977) *Mol. Pharmacol.* **13,** 442–453.
27. Johnston, G. A. R. & Willow, M. (1981) in *GABA and Benzodiazepine Receptors,* eds. Costa, E., DiChiara, G. & Gessa, G. (Raven, New York), pp.191–198.
28. Placheta, P. & Karobath, M. (1980) *Eur. J. Pharmacol.* **62,** 225–226.
29. O'Brien, R. A. & Spirt, N. M. (1980) *Life Sci.* **26,** 1441–1445.
30. Skolnick, P., Paul, S. M. & Barker, J. L. (1980) *Eur. J. Pharmacol.* **65,** 125–127.