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INTERACTION OF DEPRESSANT, CONVULSANT, AND ANTICONVULSANT BARBITURATES WITH THE [^{3}H]DIAZEPAM BINDING SITE OF THE BENZODIAZEPINE–GABA–RECEPTOR–IONOPHORE COMPLEX

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Abstract—Barbiturates with pharmacological profiles similar to that of the benzodiazepines enhance [^{3}H]diazepam binding to rat brain membranes. Diazepam binding was increased by depressant, but not by convulsant, barbiturates. (±)-Pentobarbital enhancement of diazepam binding was saturable. Besides having a direct effect, depressant barbiturates also potentiated muscimol enhancement of diazepam binding. The barbiturate-induced increase of diazepam binding was stereospecific and was blocked by γ-aminobutyric and (GABA) synaptic antagonists [(+)-bicuculline, picrotoxinin, and *t*-butyl bicyclophosphate esters] and by RO5-3663, the convulsant benzodiazepine. The ability of these antagonists to block barbiturate enhancement suggests that barbiturates may increase diazepam binding by acting on the benzodiazepine–GABA–receptor–ionophore complex.

The molecular mechanisms by which barbiturates produce their sedative-hypnotic and anticonvulsant effects have yet to be defined. Recent neuropharmacological studies suggest that barbiturates may produce some of their central nervous system (CNS) effects by facilitating γ-aminobutyric acid (GABA)-mediated inhibitory transmission [1–4]. Barbiturates have been shown to prolong the lifetime of GABA-activated Cl^- channels and to directly activate them [4]. Benzodiazepines have also been reported to potentiate Cl^--dependent GABA-mediated responses [5]. The site(s) at which barbiturates act to produce faciliation of GABAergic transmission has not been defined. Our previous studies have shown that a variety of depressant, convulsant, and anticonvulsant barbiturates potently inhibit the binding of [^{3}H]-α-dihydropicrotoxinin (DHP) to rat brain membranes [6–8]. DHP binding is distinct from the GABA recognition sites, but it appears to be associated with the benzodiazepine–GABA–receptor–ionophore complex [9, 10].

[^{3}H]Benzodiazepine binding sites in the mammalian CNS have been characterized [11–13]. GABA agonists have been reported to enhance [^{3}H]diazepam binding to rat brain membranes [14]. Recently, barbiturates have been reported to potentiate GABA enhancement of [^{3}H]diazepam binding [15] and to directly increase [^{3}H]diazepam binding [16]. In this report, we have further characterized the interaction of barbiturates and muscimol, a GABA agonist, with [^{3}H]diazepam binding to rat brain membranes.

MATERIALS AND METHODS

[^{3}H]Diazepam (68 Ci/mmole) was purchased from the New England Nuclear Corp. (Boston, MA), muscimol from Research Organics (Cleveland, OH), picrotoxinin from the Aldrich Chemical Co. (Milwaukee, WI), and other chemicals from the Sigma Chemical Co. (St. Louis, MO). The stereoisomers of hexobarbital, mephobarbital, and *N*-methyl-5-phenyl-5-propyl barbituric acid (MPPB) were gifts from Dr. J. Knabe (Saarlandes, Germany). The pentobarbital isomers were gifts from Dr. J. L. Barker (NINCDS-NIH, Bethesda, MD) and the 5-(1-3-dimethylbutyl)-5-ethyl barbituric acid (DMBB) and 5-(2-cyclohexylidene-ethyl)-5-ethyl barbituric acid (CHEB) were gifts from Eli Lilly & Co. (Indianapolis, IN) and Dr. H. Downes (University of Oregon, Portland, OR) respectively. RO5-3663 was a gift from Dr. W. E. Scott (Hoffmann-LaRoche, Nutley, NJ).

Tissue preparation. Male Sprague–Dawley rats (150–225 g) were decapitated and their brains (minus pons-medulla) were rapidly removed and placed in buffer containing 0·2 M sodium chloride and 10 mM sodium phosphate, pH 7.0 at 0–4°. The tissue was homogenized twice with a Brinkman polytron, with 5-sec bursts 15 sec apart. The homogenate was centrifuged at 1000 *g* for 10 min, the pellet was discarded, and the supernatant fraction was centrifuged at 140,000 *g* for 45 min to obtain the mitochondrial plus microsomal (P_2 + P_3) fraction. The pellet was resuspended in buffer and centrifuged at 140,000 *g* for 30 min. The pellet was washed five times by resuspension and centrifugation in the above buffer and frozen overnight. These procedures are needed to remove endogenous GABA and other inhibitory substances [17, 18]. The next day, the pellet was thawed at room temperature, pelleted, washed once, and resuspended at a protein concentration of 0.7 to 1.0 mg/ml. Protein was estimated by the method of Lowry *et al.* [19].

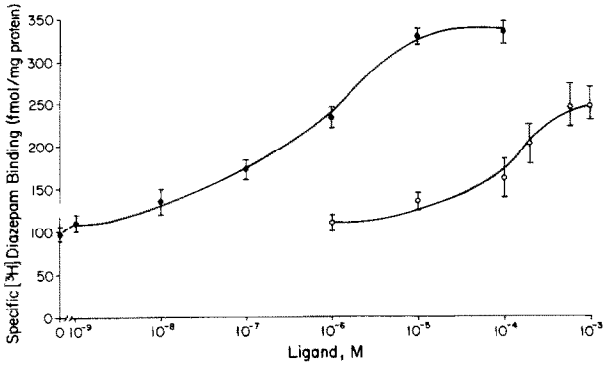

Fig. 1. Concentration-dependent enhancement of [^{3}H]diazepam binding by muscimol (●——●) and (±)-pentobarbital (○——○). Various concentrations of muscimol or pentobarbital were incubated with 1 nM [^{3}H]diazepam and membranes, as described in Materials and Methods. The curves describe enhancement over the basal diazepam binding, which was approximately 100 fmoles/mg protein. Each value is the mean ± S.D. of three experiments, each done in triplicate.

Binding assays. For the binding studies, 0.7 ml of the membrane suspension was incubated with 1 nM [^{3}H]diazepam for 30 min at 0°, with or without other drugs, in a total incubation volume of 1 ml. Following incubation, triplicate 250-μl aliquots of the incubating mixture were rapidly filtered on Whatman GF/A filters. The filters were washed with 2 × 3 ml of ice-cold buffer, dried, and counted in 5 ml toluene, containing 0.2% BBS (tissue solubilizer) (Beckman) and 5 g of 2,5-diphenyloxazole per liter. The counting efficiency determined by [^{3}H]toluene was 44 ± 2 per cent. The background was determined in the presence of 10^{-5} M diazepam. The specific binding usually represented 78 ± 10 per cent of the total binding activity. For Scatchard plots, the concentration of [^{3}H]diazepam was varied from 0.2 to 25 nM.

RESULTS

The initial experiments revealed that both muscimol and (±)-pentobarbital enhanced [^{3}H]diazepam binding significantly more to extensively washed, freeze-thawed membranes than to fresh membrane (data not shown). Furthermore, the basal [^{3}H]diazepam binding and its enhancement by muscimol and (±)-pentobarbital varied with different membrane batches, but was reproducible within the same batch.

Figure 1 shows that both muscimol and (±)-pentobarbital produced a dose-dependent enhancement of [^{3}H]diazepam binding to rat brain membranes. Muscimol was at least three orders of magnitude more potent and produced a greater increase than (±)-pentobarbital in enhancing diazepam binding. Thus, 10^{-5} M muscimol, which caused the maximal enhancement, increased the [^{3}H]diazepam binding from a basal level of 98.0 ± 4.9 fmoles/mg protein to 334 ± 7.3 fmoles/mg protein (241 per cent increase). In contrast, 10^{-3} M (±)-pentobarbital, which caused maximal enhancement, increased the [^{3}H]diazepam binding to 245 ± 20 fmole/mg protein (150 per cent increase). The (±)-pentobarbital enhancement of [^{3}H]diazepam binding was due to increased affinity (K_D) of diazepam with its binding sites. Thus, 10^{-4} M (±)-pentobarbital changed the K_D of diazepam from a control value of 9.23 ± 0.17 nM to 4.11 ± 0.22 nM ($N = 2$; $P < 0.02$) without altering the number of binding sites [control B_{max} = 1160 ± 210 fmoles/mg protein; 10^{-4} M (±)-pentobarbital-treated = 1052 ± 152 fmoles/mg protein].

Table 1. Effect of (±)-pentobarbital on muscimol enhancement of [^{3}H]diazepam binding*

Treatments	Specific [^{3}H]diazepam binding (fmoles/mg protein)	% Enhancement
Control	119.3 ± 8.7	100
+ 10^{-7} M Muscimol	182.4 ± 8.2	53
+ 10^{-5} M Muscimol	330.9 ± 6.9	177
+ 10^{-4} M (±)-Pentobarbital	152.5 ± 7.1	28
+ 5 × 10^{-4} M (±)-Pentobarbital	252.1 ± 4.5	111
+ 10^{-7} M Muscimol + 10^{-4} M (±)-pentobarbital	232.3 ± 9.1	95
+ 10^{-7} M Muscimol + 5 × 10^{-4} M (±)-pentobarbital	355.7 ± 5.8	198
+ 10^{-5} M Muscimol + 10^{-4} M (±)-pentobarbital	374.2 ± 8.8	214
+ 10^{-5} M Muscimol + 5 × 10^{-4} M (±)-pentobarbital	414.1 ± 10.7	247

* [^{3}H]Diazepam binding was studied using 1 nM [^{3}H]diazepam, as described in Materials and Methods. Muscimol or (±)-pentobarbital alone or in combination were present during the incubation. Each value is the mean ± S.D. of three experiments, each done in triplicate.

Table 2. Effect of antagonists on muscimol enhancement of [^{3}H]diazepam binding*

Treatments	Specific [^{3}H]diazepam binding (fmoles/mg protein)	% Enhancement
Control	98.2 ± 4.9	100
+ 10^{-7} M Muscimol	173.4 ± 14.6	77
+ 10^{-7} M Muscimol + 5×10^{-5} (+)-bicuculline	123.7 ± 9.7	11
+ 10^{-7} M Muscimol + 10^{-5} M RO5-3663	168.7 ± 6.7	72
+ 10^{-7} M Muscimol + 5×10^{-5} M RO5-3663	111.3 ± 8.9	13
+ 10^{-7} M Muscimol + 5×10^{-5} M picrotoxinin	175.1 ± 5.8	79
Control	112.4 ± 7.3	100
+ 10^{-5} M Muscimol	330.6 ± 9.8	194
+ 10^{-5} M Muscimol + 5×10^{-5} M (+)-bicuculline	160.8 ± 8.8	43
+ 10^{-5} M Muscimol + 10^{-5} M RO5-3663	338.9 ± 11.4	201
+ 10^{-5} M Muscimol + 5×10^{-5} M RO5-3663	221.2 ± 12.7	97
+ 10^{-5} M Muscimol + 10^{-4} M picrotoxinin	308.1 ± 9.4	174

* Rat brain membranes were incubated with 1 nM [^{3}H]diazepam without and with other ligands, as described in Materials and Methods. Each value is the mean ± S.D. of at least three experiments, each done in triplicate.

To further characterize the interaction between (±)-pentobarbital and muscimol on [^{3}H]diazepam binding, we investigated the effect of (±)-pentobarbital on muscimol enhancement. Table 1 shows that (±)-pentobarbital potentiated the muscimol-induced increase of [^{3}H]diazepam binding. Furthermore, this potentiation was additive. Submaximal concentrations of muscimol (10^{-7} M) and (±)-pentobarbital (10^{-4} M), by themselves, enhanced [^{3}H]diazepam binding, 53 and 28 per cent respectively. When present together, however, they enhanced binding 95 per cent. Similar additive enhancement was seen using 10^{-5} M muscimol in combination with 5×10^{-4} M (±)-pentobarbital. These results suggest that pentobarbital and muscimol enhance [^{3}H]diazepam binding by acting at different sites.

Table 2 shows inhibition of muscimol enhancement of [^{3}H]diazepam binding by (+)-bicuculline and the convulsant benzodiazepine, RO5-3663, but not by picrotoxinin. RO5-3663 blocked muscimol-induced increases only at concentrations greater than 10^{-5} M. At concentrations greater than 5×10^{-5} M, RO5-3663 inhibited basal [^{3}H]diazepam binding. These data are consistent with other findings, which suggest that GABA agonists enhance [^{3}H]diazepam binding by acting via the GABA receptors [14]. The inability of picrotoxinin to block muscimol enhancement is also consistent with this notion, since neither GABA nor picrotoxinin inhibits the binding of the other *in vitro* [6, 20].

Table 3 summarizes the effects of a variety of depressant, anticonvulsant, and convulsant barbiturates on [^{3}H]diazepam binding. The depressant barbiturates (±)-pentobarbital and (±)-secobarbital increased [^{3}H]diazepam binding in proportion to their dose. Furthermore, they produced similar maximal stimulations of [^{3}H]diazepam binding. The other depressant barbiturates, e.g. hexobarbital, although enhancing [^{3}H]diazepam binding, did not produce maximal effects similar to those of (±)-pentobarbital or (±)-secobarbital. At higher concentrations ($<10^{-4}$ M), (±)-hexobarbital and some of the other barbiturates inhibited basal [^{3}H]diazepam binding (Table 3). The anticonvulsant barbiturates (±)-mephobarbital and phenobarbital (data not shown) gave a partial enhancement of [^{3}H]diazepam binding. The convulsant barbiturates CHEB and (+)-MPPB did not produce significant increases in [^{3}H]diazepam binding. DMBB, which is a racemic mixture whose (+)-isomer is a convulsant and whose (−)-isomer is a depressant, also partially enhanced [^{3}H]diazepam binding. It is likely that the enhancement produced by (±)-DMBB was due to its (−)-isomer.

Table 4 shows that there appears to be stereoselectivity in the barbiturate enhancement of [^{3}H]diazepam binding. (−)-Pentobarbital, (−)-MPPB and (+)-hexobarbital were 3- to 5-fold more potent than their opposing isomers in enhancing [^{3}H]diazepam binding. This is consistent with their behavioral and neurophysiological effects.

Table 5 shows that (−)-MPPB, which is a depressant, also potentiated muscimol enhancement of [^{3}H]diazepam binding; (+)-MPPB, which is a convulsant, did not potentiate the muscimol-induced increase of [^{3}H]diazepam binding. Furthermore, the enhancement of [^{3}H]diazepam binding produced by (−)-MPPB was prevented by (+)-MPPB (data not shown).

Table 6 shows the effects of various antagonists on (±)-pentobarbital enhancement of [^{3}H]diazepam binding. The (±)-pentobarbital enhancement of [^{3}H]diazepam binding was prevented by (+)-bicuculline, bicuculline methiodide, picrotoxinin, RO5-3663 and t-butyl bicyclophosphate ester. We observed a dose-related inhibition of pentobarbital enhancement using concentrations of these antagonists that did not alter the basal [^{3}H]diazepam binding (see Table 6).

DISCUSSION

Neurophysiological and biochemical studies have shown that benzodiazepines [5, 14, 21] and barbiturates [1–4, 6–8, 20] interact with the GABA–receptor system. The GABA–receptor complex appears

Table 3. Relative enhancement of [^{3}H]diazepam binding by barbiturates*

Barbiturate	Major Activity	% Enhancement					
		10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M	5×10^{-4} M	10^{-3} M
(±)-Pentobarbital	Depressant	—	4 ± 2.0	25 ± 6	41 ± 9	110 ± 23	117 ± 19
(±)-Secobarbital	Depressant	—	6 ± 3.0	22 ± 5	45 ± 8	73 ± 15	107 ± 14
(±)-Hexobarbital	Depressant	—	21 ± 5.0	35 ± 7	I	I	—
(+)-Hexobarbital	Depressant	20 ± 4	38 ± 8.0	34 ± 9	I	—	—
(−)-Hexobarbital	Depressant	0	6 ± 6.0	12 ± 3	I	—	—
(±)-Mephobarbital	Anticonvulsant	—	4 ± 4.0	12 ± 6	I	—	—
(+)-Mephobarbital	NA†	—	5 ± 2.0	13 ± 5	I	—	—
(−)-Mephobarbital	NA	—	0	4 ± 4	I	—	—
(−)-MPPB	Depressant	—	18 ± 5.8	—	I	—	—
(+)-MPPB	Convulsant	—	6 ± 6.0	—	I	—	—
(±)-DMBB‡		16 ± 5	14 ± 6.0	6 ± 6	I	—	—
CHEB	Convulsant	—	6 ± 4.0	I	I	—	—

* [^{3}H]Diazepam binding was measured as described in Materials and Methods. Each value is the mean ± S.D. of three to six experiments, each done in triplicate. Key: (—) denotes not tested; (I) denotes inhibition of baseline.

† Not applicable.

‡ (+)-DMBB is a convulsant and (−)-DMBB is a depressant.

Table 4. Stereospecificity of barbiturate enhancement of [^{3}H]diazepam binding*

Stereoisomers	% Enhancement
10^{-4} M (−)-Pentobarbital	42 ± 6.2
10^{-4} M (+)-Pentobarbital	14 ± 5.3
10^{-6} M (+)-Hexobarbital	38 ± 8.0
10^{-6} M (−)-Hexobarbital	6 ± 6.0
10^{-6} M (−)-MPPB	18 ± 5.8
10^{-6} M (+)-MPPB	5 ± 2.9

* Each value is the mean ± S.D. of three experiments, done in triplicate.

to be composed of multicomponents, including two GABA recognition sites [20, 21], benzodiazepine binding sites [11–14] and a picrotoxinin-sensitive site associated with the GABA receptor-linked chloride ionophores [9, 10, 20]. Barbiturates and benzodiazepines potentiate GABA-mediated Cl^--dependent inhibitory transmission [1–5]. Barbiturates have also been reported to produce a direct activation of Cl^- conductance [2–4]. GABA agonists have been reported to increase the affinity of benzodiazepines for their binding sites [14]. Interaction between benzodiazepines nd Cl^- ionophores has also been demonstrated [22].

Various attempts have been made to pinpoint the

Table 5. Effect of stereoisomers of MPPB with opposing pharmacological activities on [^{3}H]diazepam binding*

Treatment	Specific [^{3}H]diazepam binding (fmoles/mg protein)	% Enhancement
Control	93.1 ± 4.2	100
+ 10^{-6} M (−)-MPPB	112.5 ± 3.7	21
+ 10^{-6} M (+)-MPPB	98.6 ± 4.5	6
+ 10^{-5} M Muscimol	257.7 ± 7.3	177
+ 10^{-5} M Muscimol + 10^{-6} M (−)-MPPB	274.8 ± 5.4	195
+ 10^{-5} M Muscimol + 10^{-6} M (+)-MPPB	233.7 ± 9.6	151

* Muscimol and the (−)- and (+)-isomers of MPPB were incubated alone or in combination with 1 nM [^{3}H]diazepam, as described in Materials and Methods. Each value is the mean ± S.D. of two experiments, each done in triplicate.

Table 6. Effect of antagonists on (±)-pentobarbital enhancement of [^{3}H]diazepam binding*

Treatment	% Inhibition of 5×10^{-4} M (±)-pentobarbital enhancement
Control (5×10^{-4} M (±)-pentobarbital)	100
5×10^{-4} M (±)-Pentobarbital	
+ 5×10^{-6} M (+)-Bicuculline	18 ± 4.2
+ 10^{-5} M (+)-Bicuculline	42 ± 15.0
+ 5×10^{-5} M (+)-Bicuculline	65 ± 9.7
+ 10^{-5} M Bicuculline methiodide	40 ± 8.9
+ 5×10^{-5} M Bicuculline methiodide	57 ± 9.4
+5×10^{-6} M Picrotoxinin	4 ± 4.0
+ 10^{-5} M Picrotoxinin	40 ± 10.6
+ 5×10^{-5} M Picrotoxinin	57 ± 11.3
+ 10^{-4} M Picrotoxinin	73 ± 9.8
+ 10^{-5} M RO5-3663	23 ± 5.6
+ 2×10^{-5} M RO5-3663	48 ± 6.7
+ 5×10^{-5} M RO5-3663	72 ± 7.7
+ 10^{-5} M t-Butyl bicyclophosphate esters	38 ± 5.9

* Rat brain membranes were incubated with 1 nM diazepam and 5×10^{-4} M (±)-pentobarbital alone or in combination with other antagonists, as indicated. (±)-Pentobarbital alone enhanced basal [^{3}H]diazepam binding by 70–120 per cent, depending upon the membrane batch. Antagonist concentrations listed in the table did not alter the basal [^{3}H]diazepam binding. Higher concentrations of some antagonists did decrease basal [^{3}H]diazepam binding. Each value is the mean ± S.D. of four to six experiments, each done in triplicate.

site at the benzodiazepine–GABA–receptor–ionophore complex that may be involved in barbiturate action. Barbiturates do not appear to affect GABA synthesis, uptake or release [23]. Although previous studies failed to demonstrate an interaction between barbiturates and GABA receptor binding sites [6, 20, 24, 25], a recent study has shown that pentobarbital increased the affinity of the GABA binding sites [26]. It is interesting that this change in affinity was prevented by picrotoxin. Our own studies have demonstrated that a variety of depressant, convulsant, and anticonvulsant barbiturates, although having no effect on GABA receptor binding, inhibit the binding of [^{3}H]DHP to rat brain membranes [6–8, 20]. DHP binds at a site distinct from the GABA recognition sites. Several lines of evidence support the notion that DHP binding at the GABA–receptor–ionophore complex appears to be related to the pharmacological action of picrotoxin [9, 10] and that this site is a target for depressant and convulsant drug action [6–9, 20].

In the present study, we find that both muscimol and pentobarbital produce a dose-related enhancement of diazepam binding. Both of these ligands produce an increase in the affinity without altering the density of diazepam binding sites. These results are in agreement with the findings of Tallman *et al.* [14] and Leeb-Lundberg *et al.* [16]. Our recent studies have shown that ethanol, which has a pharmacological profile similar to that of barbiturates and benzodiazepines, also enhances diazepam binding and potentiates muscimol enhancement of diazepam binding [27]; (M. K. Ticku and T. Burch, unpublished results).

Our results also show that pentobarbital does not give the same maximum stimulation of diazepam binding as seen with muscimol. Furthermore, the effects of pentobarbital and muscimol on enhancing diazepam binding were additive, using concentrations that produce both submaximal and maximal enhancement. These results suggest that pentobarbital may enhance diazapam binding by acting at a site different from the muscimol (i.e. GABA) binding site. Further support for this is provided by the finding that muscimol enhancement was blocked by (+)-bicuculline but not by picrotoxinin (Table 2). (±)-Pentobarbital enhancement was blocked by both of these ligands. RO5-3663, a convulsant benzodiazepine that has no effect on benzodiazepine binding, also blocked muscimol enhancement, albeit at high concentrations. Recently, it has been reported that RO5-3663 inhibits DHP binding competitively [28]. Our studies have also shown that RO5-3663 inhibits DHP binding both to membranes and, in soluble form, to rat brain membranes [29, 30].

The diazepam binding was enhanced by depressant barbiturates but not by the convulsant barbiturates CHEB and (+)-MPPB. DMBB, which is a racemic mixture, produced a partial enhancement at 10^{-7} to 10^{-6} M. At higher concentrations, it inhibited the baseline diazepam binding. It is reasonable to assume that the enhancement produced by DMBB was due to the (−)-isomer, which is a depressant [31]. The anticonvulsant barbiturates phenobarbital (data not shown) and mephobarbital produced a partial enhancement of [^{3}H]diazepam binding.

The barbiturate enhancement of diazepam binding appears to have been sterospecific. (−)-Pentobarbital and (−)-MPPB, which are CNS depressants, were approximately 3-fold more potent than their (+)-isomers in enhancing diazepam binding. These results are in agreement with neurophysiological studies. It has been reported that (−)-pentobarbital is more potent than (+)-pentobarbital in potentiating GABA responses [4]. Similarly, (+)-hexobarbital is a better depressant than (−)-hexobarbital [32]. In the case of MPPB, it is known that the (−)-isomer is a depressant and that the (+)-isomer is a convulsant [33]. These stereoisomers inhibit DHP binding in a similar fashion [7, 8]. Both depressant and convulsant barbiturates inhibit DHP binding. Only depressant barbiturates enhance [^{3}H]diazepam binding.

It is interesting to note that pentobarbital enhancement of diazepam binding was blocked by ligands that are GABA synaptic antagonists. Thus, (+)-bicuculline and bicuculline methiodide, which act at the GABA recognition site, inhibited pentobarbital enhancement of diazepam binding.

The ability of (+)-bicuculline to block pentobarbital enhancement of diazepam binding to membranes in the absence of GABA was surprising. We have observed recently that (+)-bicuculline also blocks the pentobarbital enhancement of diazepam binding to soluble receptors ([34]; W. C. Davis and M. K. Ticku, unpublished results). Recently, it has been reported that a purine derivative (EMD 28422) that does not appear to bind at the GABA recognition level nonetheless enhances diazepam binding, and this effect is blocked by bicuculline [35]. These results suggest that the GABA recognition site may be involved in modulating the function of benzodiazepine receptor sites. It is feasible that (+)-bicuculline, by binding at the GABA recognition site, prevents the conformation change induced by ligands like pentobarbital and EMD 28422, which bind at other sites.

Pentobarbital enhancement of diazepam binding was also blocked by the ligands picrotoxinin, t-butyl bicyclophosphate and RO5-3663. These ligands are convulsants, GABA synaptic antagonists, and appear to act at the picrotoxinin binding component of the benzodiazepine–GABA–receptor complex [6–9, 20, 28, 29]. Barbiturates inhibit DHP binding, and ligands that act at the DHP site prevent pentobarbital enhancement; it is reasonable to speculate that barbiturates enhance diazepam binding by acting at the picrotoxinin component of the benzodiazepine–GABA–receptor–ionophore complex.

At present, it is not feasible to relate the barbiturate enhancement of diazepam binding to either depressant or anticonvulsant actions. It is possible that the increase in diazepam binding may be related to the CNS depressant action, as suggested [16]. It may be noted, however, that all depressant barbiturates did not give the same maximum enhancement. Using Ariën's concept of intrinsic activity [36], some depressant barbiturates appear to be partial agonists in this system, since they do not give the same maximum effect. Furthermore, the physiological significance of enhanced diazepam binding has yet to be established. Nonetheless, the ability of barbitu-

rates [1–4] and ethanol [37] to facilitate GABAergic transmission and to enhance diazepam binding at the benzodiazepine–GABA–receptor–ionophore complex must be related to some of their effects in the CNS.

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