0026-895X/92/061124-06$03.00/0

MOLECULAR PHARMACOLOGY, 41:1124-1129

Functionally Relevant γ-Aminobutyric Acid$_A$ Receptors: Equivalence between Receptor Affinity (K_d) and Potency (EC_{50})?

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Received July 11, 1991; Accepted March 30, 1992

SUMMARY

Many neurotransmitter receptors bind agonists with high affinity (K_d in the nanomolar range), whereas micromolar concentrations of the same agonists are required to elicit a functional effect. We have identified low affinity agonist binding sites for the γ-aminobutyric acid$_A$ (GABA$_A$) receptor-chloride channel under conditions normally used in $^{36}Cl^-$ uptake assays (a measure of receptor function). The GABA$_A$ agonist [^{3}H]muscimol bound to a population of receptors with a K_d (2 μM) similar to its EC_{50} value for $^{36}Cl^-$ uptake. Binding was inhibited by the GABA agonist 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol and by the GABA antagonist bicuculline methiodide. A reduction in the number of [^{3}H]muscimol binding sites (B_{max}) by a thiol-modifying reagent produced a corresponding decrease in the E_{max} for muscimol. The benzodiazepine diazepam enhanced the potency of muscimol in ion flux experiments but did not alter the affinity of [^{3}H]muscimol binding sites. We propose that benzodiazepines enhance GABAergic function by increasing receptor-ion channel coupling, rather than by increasing GABA$_A$ receptor affinity. These studies suggest that the study of physiologically relevant (low affinity) binding sites is necessary when examining regulation of receptors by cellular processes, drugs, and disease.

Receptor binding assays have been used for more than 20 years to measure the characteristics of binding of neurotransmitters, hormones, and drugs to specific proteins that exist within the cell membrane or cytoplasm. The two major parameters obtained from these studies are the K_d, which indicates the affinity of the receptor for a specific ligand, and the B_{max}, which indicates the density of sites labeled by the ligand in a particular tissue. These parameters have provided important information concerning the characteristics of a receptor system and the regulation of receptors by intracellular processes, drug administration, and disease. One of the hallmarks of receptor binding assays is the measurement of sites with high affinity; the K_d is typically in the low nanomolar range for most neurotransmitters and their agonists (1–3). In contrast, micromolar concentrations of the same neurotransmitters and agonists are usually required to produce an effect in assays of receptor function; potencies, reflected by the EC_{50}, are usually in the micromolar range (4–6). *In vivo*, where micromolar to millimolar concentrations of neurotransmitter in the synapse can occur, neurotransmitters bind to receptors with a high rate of dissociation (low affinity), to maintain normal synaptic transmission. Attempts to explain the 1000-fold discrepancy between the K_d and the EC_{50}, *in vitro*, have been made by several investigators (7, 8), but a consensus has not been reached. The most likely explanation is that the conditions for assaying receptor function and binding characteristics are different. Functional studies are usually performed under conditions that approximate physiologic conditions, whereas binding studies are usually performed under conditions established by the investigator to optimize the ligand binding signal. Thus, the following question has been posed: what is the functional significance of the high affinity receptor, measured *in vitro*? For some receptors, such as the nicotinic acetylcholine and GABA$_A$ receptors, investigators have suggested that the high affinity sites reflect the desensitized state of the receptor (8–11). However, many agree that the high affinity state of the receptor is probably not responsible for the response elicited by agonist occupation of the receptor (8–13), although certainly it may exist *in vivo*.

To address this issue, we sought to measure "functional" agonist binding sites for the GABA$_A$ receptor. GABA is the major inhibitory neurotransmitter in brain and gates a chloride channel (for review, see Ref. 14). The literature reveals that GABA, as well as its agonist muscimol, bind to GABA$_A$ receptors with high affinity; K_d values have been measured in the 3 nM to 500 nM range (9, 15–19). In addition, several investigators

This work was supported by a National Science Foundation Predoctoral Fellowship to P.P.E. and by National Institutes of Health Grant NS 24577 to R.D.S.

ABBREVIATIONS: K_d, equilibrium dissociation constant; GABA, γ-aminobutyric acid; EC_{50}, concentration of compound resulting in 50% of maximal response; B_{max}, maximal density of binding sites under equilibrium binding conditions; E_{max}, maximal response to a given compound; NEM, *N*-ethylmaleimide; THIP, 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

have suggested the presence of even lower affinity sites, with a K_d of >1 μM (9, 17, 18). These low affinity sites, when occupied by agonists, most likely mediate the functional responses of $GABA_A$ receptors, as assayed by ion flux and electrophysiologic techniques. The potencies for GABA and its agonist muscimol to stimulate uptake of $^{36}Cl^-$ into brain vesicles and to inhibit neuronal firing are in the low micromolar range (5–15 μM) (6, 13, 20–22). Even lower potencies can be obtained when GABA-mediated ion flux is measured on a millisecond time scale (23). The goal of the present study was to identify functionally relevant $GABA_A$ recognition sites labeled with [^{3}H]muscimol, by performing the binding assay under the conditions used to measure $GABA_A$ receptor function. Our results suggest that the binding sites identified under these conditions are of low affinity (micromolar K_d) and, when occupied by muscimol, are responsible for opening the GABA-gated chloride channel.

Experimental Procedures

Materials. Experiments were performed on adult male Sprague-Dawley rats (150–225 g) obtained from Zivic-Miller Laboratories, Inc. (Zelienople, PA). Chlorine-36 (20–26 MBq/mmol) was obtained from New England Nuclear (Boston, MA). [*methylamine*-^{3}H]Muscimol (5–20 Ci/mmol) was obtained from Amersham Corp. (Arlington Heights, IL). Muscimol, NEM, GABA, picrotoxin, and other chemicals were obtained from Sigma Chemical Company (St. Louis, MO).

Preparation of synaptoneurosomes. Synaptoneurosomes were prepared from adult male Sprague-Dawley rats as previously described by Schwartz *et al.* (6), with minor modifications. Brains were removed rapidly after decapitation and placed immediately in ice-cold synaptoneurosome buffer (pH 7.4) containing 10 mM glucose, 20 mM HEPES, 9 mM Tris, 118 mM NaCl, 4.7 mM KCl, 1.18 mM $MgSO_4$, and 1.0 mM $CaCl_2$. Cerebral cortex was dissected free of white matter and homogenized by hand (five strokes) in 7 volumes (w/v) of buffer, using a glass-glass homogenizer. The homogenate was diluted with 30 ml of buffer/g of tissue and filtered by gravity through three layers of nylon mesh (160 μm; Tetko Inc., Elmsford, NY). The filtrate was then gently filtered through a 10-μm Mitex filter (Millipore, Bedford, MA) and centrifuged at 1000 × *g* for 15 min. The supernatant was discarded, and the pellet was gently resuspended and recentrifuged (1000 × *g*, 15 min). The final pellet was resuspended to a protein concentration of 10 mg/ml for $^{36}Cl^-$ uptake experiments or 2 mg/ml for binding experiments. The filtration steps described above effectively remove large cellular debris, oligodendrocytes, and intact neurons, resulting in a relatively homogeneous population of both pre- and postsynaptic membrane vesicles ("synaptoneurosomes"; for details, see Ref. 24).

$^{36}Cl^-$ uptake assay. $^{36}Cl^-$ uptake was measured as previously described by Schwartz *et al.* (6). Synaptoneurosomes (0.5–1.0 mg of protein) were preincubated with drugs for 5 min at 30° before the simultaneous addition of 0.5 μCi of $^{36}Cl^-$ and 50 μM muscimol. Basal uptake of $^{36}Cl^-$ was determined in the absence of muscimol. $^{36}Cl^-$ uptake was terminated after 5 sec by dilution of the assay mixture with 5 ml of ice-cold buffer, containing 100 μM picrotoxin, and filtration by vacuum through a glass fiber filter (no. 30; Schleicher & Schuell, Keene, NH). The filters were washed two times with 5 ml of cold buffer (containing picrotoxin) and counted, by liquid scintillation counting, for retained radioactivity.

Low affinity [^{3}H]muscimol binding assay. Synaptoneurosomes (0.1 mg of protein) were preincubated, in Eppendorf tubes containing synaptoneurosome buffer and drugs, for 5 min at 30° before the addition of [^{3}H]muscimol (10, 20, or 30 nM) with increasing concentrations of unlabeled muscimol (0–30 μM). Nonspecific binding was defined in the presence of 1 mM GABA. The incubation was terminated after 30 min by centrifugation (13,000 × *g*, 0–4°), and the supernatant was removed. Pellets were washed two times with 500 μl of cold buffer, solubilized in 1 ml of Protosol (New England Nuclear) for 45 min at 40°, and left to sit overnight. On the next day, 10 ml of scintillation mixture (toluene, 2,5-diphenyloxazole, and 1,4-bis-[2-(5-phenyloxazolyl)]benzene were added, and vials were counted by using liquid scintillation counting.

Measurement of GABA accumulation in synaptoneurosomes by high performance liquid chromatography. Synaptoneurosomes were incubated with or without NEM (200 μM) for 30 min at 30°. The incubation was terminated by centrifugation (10,000 × *g*, 15 min at 0–4°), and the supernatant from each tube was transferred to a microfilterfuge tube (0.45-μm; Rainin, Woburn, MA). After recentrifugation (10,000 × *g*, 4 min at 0–4°), the filtrate was assayed for GABA released, using reverse phase high performance liquid chromatography (25). Samples were diluted, derivatized with *o*-phthaldialdehyde, and, 90 sec later, injected onto a 3-μm C-18 reverse phase column (MacMod Analytical, Chadds Ford, PA). Cysteic acid was included in each sample as an internal standard. The mobile phase consisted of a linear gradient of 80% 0.04 M NaH_2PO_4 (pH 5.7) and 20% methanol. *o*-Phthaldialdehyde-derivatized GABA was detected by fluorescence spectroscopy, and peak heights were compared with the internal standard. The limit of sensitivity was 50 fmol.

Protein determination. Protein concentrations were determined by the method of Lowry *et al.* (26), using bovine serum albumin as standard.

Data analysis. The EC_{50} and E_{max} values were calculated using nonlinear curve fitting of concentration-response data (PCS version 4.0). K_d and B_{max} values were obtained, using either Scatchard analyses or nonlinear curve-fitting programs (Lundon Software), from data corrected for nonsaturable binding. Nonspecific binding was subtracted from total binding to obtain specific binding. Nonsaturable specific binding was defined as the horizontal linear portion of the Scatchard plot. The bound/free value (*y*-intercept) for this line was subtracted from each point, and the data were replotted for determination of K_d and B_{max} values by Scatchard analysis. Student's *t* tests were used for statistical comparisons of two groups of means. A probability level (p) of <0.05 was considered to be statistically significant.

Results and Discussion

$GABA_A$ receptor function was measured using muscimol-induced $^{36}Cl^-$ uptake into synaptoneurosomes from rat cerebral cortex (6). Synaptoneurosomes are intact brain vesicles consisting of resealed pre- and postsynaptic membranes; the preparation has been characterized morphologically and biochemically (24). Muscimol stimulated $^{36}Cl^-$ uptake into synaptoneurosomes in a concentration-dependent manner, with an EC_{50} of 9.9 ± 1.1 μM (six experiments). Maximal muscimol-induced $^{36}Cl^-$ uptake (E_{max}) was 79.8 ± 4.9 nmol of $^{36}Cl^-$/mg of protein (six experiments). [^{3}H]Muscimol binding studies were carried out in the intact synaptoneurosomes using the same buffer (modified Krebs-Henseleit) and temperature (30°) used in the $^{36}Cl^-$ flux assay. Saturation experiments were performed using unlabeled muscimol (0.01–30 μM) and [^{3}H]muscimol (10 or 20 nM). Specific [^{3}H]muscimol binding ranged from 56 to 67% of total binding; nonspecific binding was defined in the presence of 1 mM GABA or 200 μM bicuculline methiodide. A Scatchard analysis of saturation binding data indicated a population of saturable sites (Fig. 1, *inset*) and a population of nonsaturable sites for [^{3}H]muscimol (Fig. 1). Nonsaturable binding persisted at concentrations of muscimol up to 10 mM (data not shown). This population of sites could represent uptake of [^{3}H]muscimol by the GABA uptake system, because the assay is performed at 30° in the presence of 118 mM NaCl and the population is removed by the addition of the GABA uptake inhibitor nipecotic acid (data not shown). Although muscimol is a poor substrate for the GABA uptake carrier (K_m = 1 mM) (27),

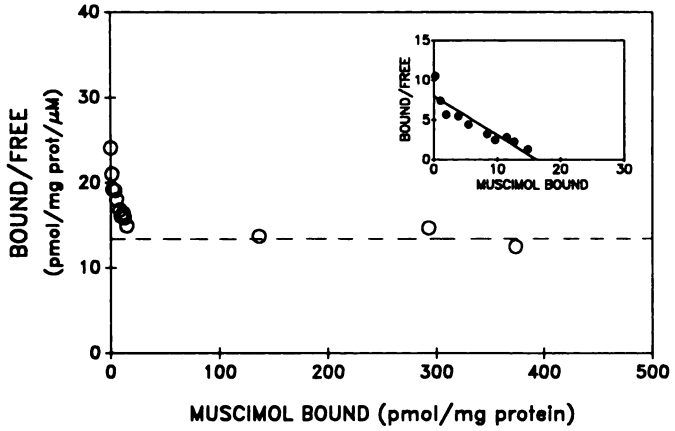

Fig. 1. Scatchard analysis of low affinity [^{3}H]muscimol binding to synaptoneurosomes. Synaptoneurosomes were incubated for 30 min with 0.01–30 μM muscimol containing 10 or 20 nM [^{3}H]muscimol. Nonspecific binding (33–44%) was defined in the presence of 1 mM GABA and was subtracted from total binding, to obtain specific binding. *Dashed line*, specific but nonsaturable [^{3}H]muscimol binding, which was subtracted from all points, to obtain a linear Scatchard plot (*inset*) from which K_d and B_{max} were determined. K_d and B_{max} values were 2.1 ± 0.1 μM and 20.4 ± 1.2 pmol/mg of protein, respectively (14 experiments).

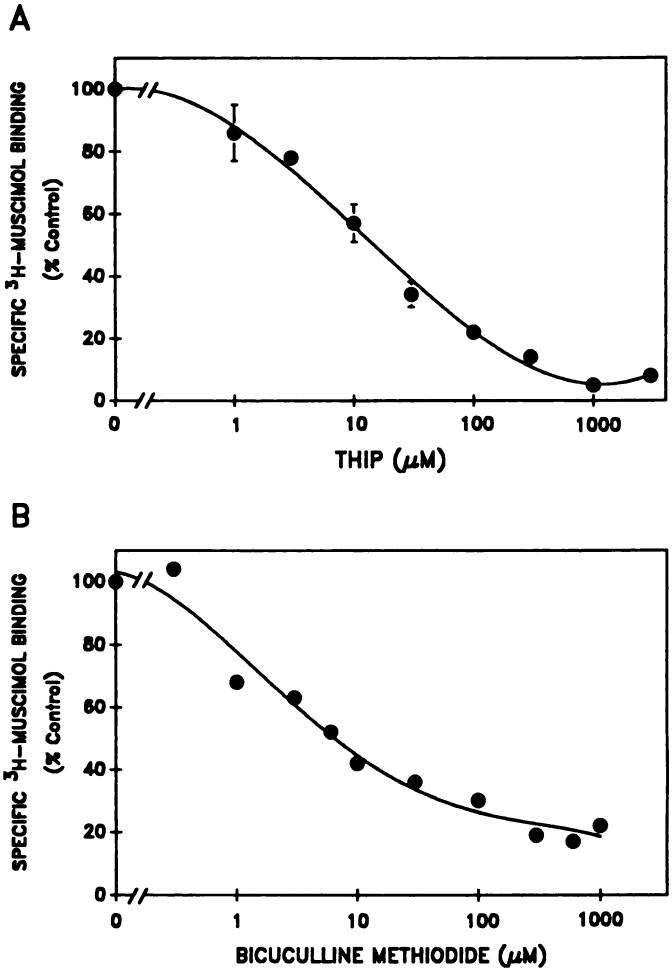

Fig. 2. Competition of [^{3}H]muscimol binding with THIP and bicuculline methiodide. Synaptoneurosomes were incubated with 50 nM [^{3}H]muscimol and various concentrations of THIP (A) or bicuculline methiodide (B). Nonsaturable [^{3}H]muscimol binding (59%) was subtracted before analysis of competition curves. Concentration-response data from three experiments (in triplicate) were combined and analyzed using a nonlinear curve-fitting program, to obtain EC_{50} values of 9.1 and 2.5 μM, respectively.

uptake of [^{3}H]muscimol may occur at the highest concentrations used here (10 and 30 μM). The contribution of the nonsaturable sites was subtracted from the total binding at all points, in order to reveal a saturable population of binding sites (Fig. 1, *inset*) with a K_d of 2.1 ± 0.1 μM and a B_{max} value of 20.4 ± 1.2 pmol/mg of protein (14 experiments). It is unlikely that these sites reflect [^{3}H]muscimol uptake, for the following reasons: 1) the nonsaturable binding component has been subtracted, 2) the K_m for muscimol uptake is 3 orders of magnitude greater than the K_d for these sites, and 3) nonspecific binding, defined by GABA (1 mM), was unchanged when GABA was added to the incubation medium 10 min after the initiation of [^{3}H]muscimol binding (data not shown). The comparable values of the K_d (2.1 ± 0.1 μM) and the EC_{50} (9.9 ± 1.1 μM) for muscimol suggest that occupation of these [^{3}H]muscimol binding sites by muscimol is responsible for inducing $^{36}Cl^-$ uptake.

Pharmacologic characterization of low affinity [^{3}H]muscimol binding revealed that both THIP (a $GABA_A$ agonist) and bicuculline methiodide (a competitive $GABA_A$ antagonist) inhibit the binding of [^{3}H]muscimol (50 nM) to synaptoneurosomes (Fig. 2). The EC_{50} for THIP inhibition of [^{3}H]muscimol binding was 9.1 μM, similar to its potency for inducing $^{36}Cl^-$ uptake in synaptoneurosomes (28). The EC_{50} for bicuculline methiodide inhibition of [^{3}H]muscimol binding was 2.5 μM, similar to its potency for inhibition of muscimol-induced $^{36}Cl^-$ uptake in synaptoneurosomes (EC_{50} = 1.5 μM) and in cultured spinal cord neurons (29).

To examine the relationship of [^{3}H]muscimol sites to $GABA_A$ receptor function, binding and $^{36}Cl^-$ uptake assays were performed on synaptoneurosomes pretreated with NEM, a reagent that covalently alters thiol groups associated with many receptors (30). Pretreatment of synaptoneurosomes for 5 min with 200 μM NEM decreased the E_{max} for muscimol-induced $^{36}Cl^-$ uptake by 46%, without significantly altering the EC_{50} (Fig. 3; Table 1). Similarly, in the [^{3}H]muscimol binding assay, NEM decreased the B_{max} by 55%, without altering the K_d of the remaining [^{3}H]muscimol binding sites (Fig. 4; Table 1). It should be noted that NEM, as a covalent modifying agent, is not selective for receptors and might interfere with other processes, such as neurotransmitter uptake. In fact, NEM and other thiol-modifying reagents have been reported to produce extravesicular GABA accumulation in brain vesicles (31, 32). However, previous studies indicated that NEM decreased the number (B_{max}) of [^{3}H]benzodiazepine binding sites associated with the $GABA_A$ receptor under conditions where uptake does not occur (33). Furthermore, it is unlikely that significant GABA release or inhibition of GABA uptake occurred here, because there was no change in 1) the K_d for [^{3}H]muscimol binding, 2) the EC_{50} for muscimol-induced $^{36}Cl^-$ uptake, or 3) basal $^{36}Cl^-$ uptake. To address this issue directly, we measured the accumulation of GABA in the extravesicular space after exposure to NEM. When synaptoneurosomes were incubated with 200 μM NEM, as described in the binding assays, there was no difference in GABA accumulation (203 versus 189 pmol/mg of protein, in the absence and presence of NEM, respectively). This finding rules out the possibility that 1) NEM inhibits GABA uptake under these conditions or 2) NEM decreases [^{3}H]muscimol binding indirectly, via accumulation of GABA. Although we have not demonstrated covalent modification of

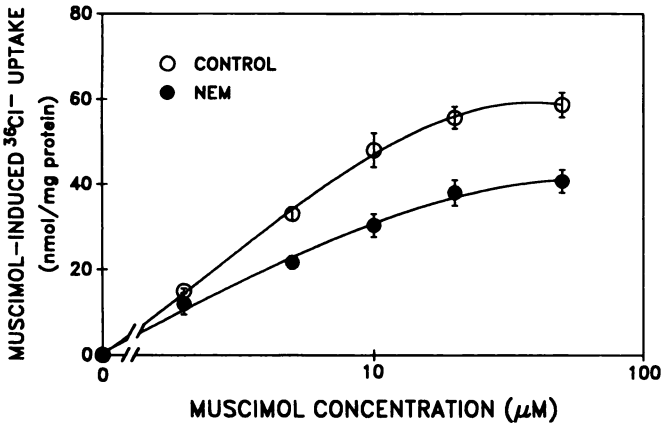

Fig. 3. Inhibition of muscimol-induced $^{36}Cl^-$ uptake in synaptoneurosomes pretreated with NEM. Synaptoneurosomes were preincubated for 10 min with buffer (○) or with 200 μM NEM (●) before measurement of $^{36}Cl^-$ uptake, as described in the text. EC_{50} and E_{max} values determined from linear transformation of the data are listed in Table 1. Basal $^{36}Cl^-$ uptake values in the absence and presence of NEM were 785 ± 29 and 907 ± 40 nmol/mg of protein, respectively. Data are the mean ± standard error from three experiments.

TABLE 1

Effect of NEM and diazepam on [^{3}H]muscimol binding and muscimol-induced $^{36}Cl^-$ uptake in synaptoneurosomes

Data from binding (five experiments) and $^{36}Cl^-$ uptake (three experiments) experiments were analyzed separately.

	[^{3}H]Muscimol binding		Muscimol-induced $^{36}Cl^-$ uptake	
	K_d	B_{max}	EC_{50}	E_{max}
	μM	pmol/mg of protein	μM	nmol/mg of protein
Control	2.1 ± 0.1	25.6 ± 4.0	8.7 ± 1.0	83.8 ± 7.0
NEM	2.2 ± 0.6	11.6 ± 2.6[a]	5.2 ± 0.8	45.2 ± 2.9[a]
Control	2.1 ± 0.4	17.7 ± 1.7	11.2 ± 1.7	75.8 ± 5.9
Diazepam	2.3 ± 0.3	19.0 ± 2.1	4.7 ± 0.6[b]	64.5 ± 1.7

[a] $p < 0.01$, compared with respective controls (Student's t test).
[b] $p < 0.05$, compared with control (Student's t test).

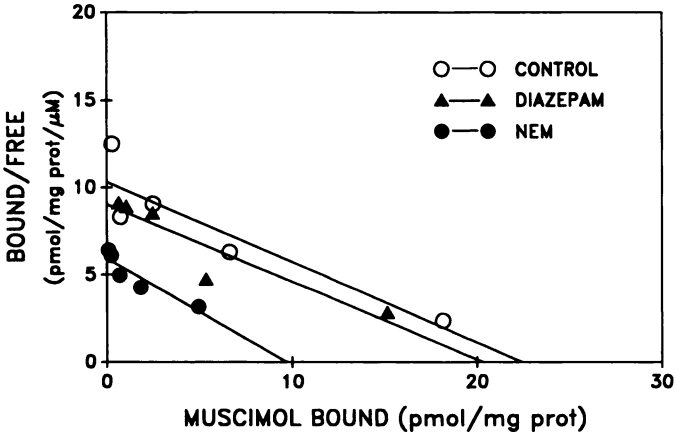

Fig. 4. Effects of NEM and diazepam on [^{3}H]muscimol binding. Synaptoneurosomes were preincubated for 5 min with NEM (200 μM) or diazepam (10 μM) before a 30-min incubation with muscimol (0.01–30 μM) containing 10 or 20 nM [^{3}H]muscimol. K_d and B_{max} values were calculated from Scatchard plots, as described for Fig. 1, and are listed in Table 1. Data from a single experiment are representative of five experiments.

the GABA$_A$ receptor by NEM, the selective effect of NEM on maximal muscimol responses and binding density, rather than potency and affinity, suggests the disruption of binding and function at a common locus.

The assay conditions that have been used in most GABA agonist binding studies (frozen/thawed, well washed, membrane fragments; 0–4°; 50 mM Tris buffer) have favored binding to the higher affinity sites (17). In the present study, we demonstrate that low affinity sites for [^{3}H]muscimol can be measured, although we cannot rule out unequivocally the measurement of [^{3}H]muscimol uptake. (Any compound that inhibits the uptake mechanism in an intact preparation will cause the accumulation of GABA, which will inhibit [^{3}H]muscimol binding.) The major factors that might contribute to the measurement of a micromolar K_d are 1) the increased temperature (30°), 2) the presence of endogenous GABA, and 3) the use of an intact vesicular preparation. In the first case, binding studies performed at temperatures greater than 0–4° result in identification of [^{3}H]muscimol binding sites in the medium to low affinity range (17, 19). Second, although the endogenous GABA levels are higher than would be present in a frozen-thawed, well washed, lysed membrane preparation, an active uptake pump for GABA keeps the extravesicular GABA level in synaptoneurosomes extremely low (approximately 140–200 pmol/mg of protein or 30–40 nM in binding experiments). In fact, the presence of 30 nM extravesicular GABA does not contribute to the low affinity K_d for [^{3}H]muscimol measured here. In the third case, other endogenous factors that are absent from high affinity membrane binding preparations may contribute to the measurement of a low affinity K_d in synaptoneurosomes. Because the presence of endogenous factors (including GABA) in our intact vesicular preparation reflects their presence *in vivo*, the binding constants obtained under our conditions may be a more accurate estimate of the receptor characteristics *in vivo* than are those obtained under high affinity conditions *in vitro*.

When measuring muscimol-induced $^{36}Cl^-$ uptake over a 5-sec time period, we have obtained EC_{50} values that range between 2 and 10 μM. According to calculations by Kardos and Cash (23) of GABA-induced $^{36}Cl^-$ uptake, the EC_{50} does not change appreciably at longer uptake times. In contrast, they reported that the EC_{50} for GABA increases as the uptake time for $^{36}Cl^-$ is reduced to milliseconds. In theory, the active GABA uptake system will cause a greater increase in the GABA EC_{50} during shorter assay times, relative to longer ones. It is not known whether the EC_{50} for muscimol also increases at shorter flux times, despite its poor affinity for the GABA uptake carrier. Because we cannot measure $^{36}Cl^-$ uptake in the millisecond time scale, using the manual techniques described here, the EC_{50} values that we obtained for muscimol-induced $^{36}Cl^-$ uptake may be an underestimate of the true EC_{50} that occurs *in vivo*.

The significance of measuring low affinity binding sites is readily apparent when considering the mechanism(s) of action of agents that alter GABA$_A$ receptor function. An important example is the benzodiazepines, which possess anxiolytic, anticonvulsant, and sedative actions. Both biochemical and electrophysiologic studies indicate that GABA$_A$ receptor function is enhanced by benzodiazepines (20–22). Many investigators have suggested that benzodiazepine-enhanced GABA$_A$ receptor function, indicated by a leftward shift of the GABA concentration-response curve, reflects an increased GABA$_A$ receptor affinity produced by benzodiazepines (34, 35). However, experi-

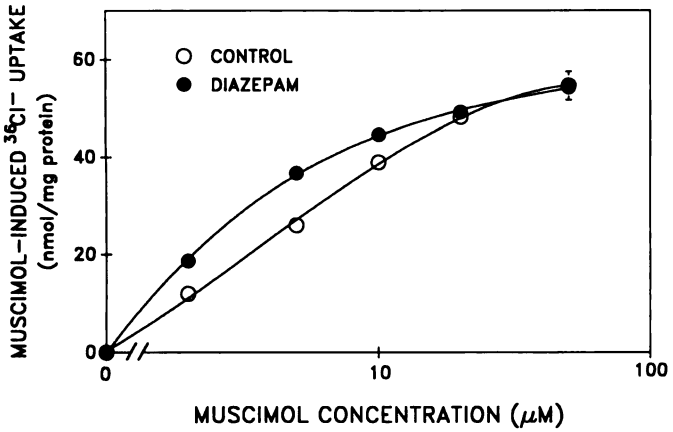

Fig. 5. Enhancement of muscimol-induced $^{36}Cl^-$ uptake by diazepam. Synaptoneurosomes were preincubated with buffer (○) or 10 μM diazepam (●) before measurement of muscimol-stimulated $^{36}Cl^-$ uptake, as described in the text. EC_{50} and E_{max} values are listed in Table 1. Data are the mean ± standard error of three experiments; error bars were omitted when smaller than the symbol.

mental evidence for enhanced receptor affinity is weak; a few studies have revealed small increases in $GABA_A$ receptor affinity produced by benzodiazepines (36, 37), whereas most fail to demonstrate this effect (3, 17, 38). It has been suggested previously that enhanced receptor affinity might be revealed under conditions where low affinity $GABA_A$ receptors are measured, because they are more likely to be the functional sites. We tested this hypothesis under our conditions, in which low affinity $GABA_A$ receptors are measured. Preincubation of synaptoneurosomes with diazepam (10 μM, 10 min) shifted the concentration-response curve for muscimol-induced $^{36}Cl^-$ uptake to the left, decreasing the EC_{50} from 11.2 ± 1.7 μM to 4.7 ± 0.6 μM (Fig. 5; Table 1). Under the same conditions, diazepam did not change the number or affinity of [^{3}H]muscimol binding sites (Fig. 4; Table 1). Enhancement of muscimol-induced $^{36}Cl^-$ uptake by diazepam confirms studies showing that benzodiazepines potentiate GABAergic function by decreasing the agonist EC_{50} (20–22). In contrast to the postulated mechanism, diazepam failed to alter low affinity [^{3}H]muscimol binding to $GABA_A$ receptors. Electrophysiologic studies clearly demonstrate that benzodiazepines increase chloride channel opening in response to GABA (34, 39). Twyman *et al.* (34) suggest that a benzodiazepine-induced increase in $GABA_A$ receptor agonist binding affinity accounts for the increase in frequency of channel opening. Alternatively, we propose that benzodiazepines enhance $GABA_A$ receptor function by enhancing receptor-ion channel coupling and not by increasing the affinity of the receptor for the agonist.

In conclusion, "equivalence" between the parameters for receptor ligand affinity (K_d) and potency (EC_{50}) can be achieved when the conditions for their assay are the same. Generally, this requires an intact cell system, such as intact vesicular preparations or cultured cells. However, uptake of the radioligand (or binding to an uptake carrier) is a potential problem when intact preparations are used and must be controlled for. In most cases, the receptor binding assay will require conditions used in functional assays to identify the population of receptors that produces a biologic response. This is most important when studying the regulation of receptors by cellular events, drugs, and disease.

Acknowledgments

We gratefully acknowledge the excellent technical assistance of Ms. Xiao Yu.

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