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STIMULATION OF BENZODIAZEPINE BINDING TO RAT BRAIN MEMBRANES AND SOLUBILIZED RECEPTOR COMPLEX BY AVERMECTIN B_{1a} AND γ-AMINOBUTYRIC ACID

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The binding of [^{3}H]flunitrazepam to benzodiazepine receptors in synaptic membranes and a digitonin-solubilized receptor fraction of rat brain is increased by avermectin B_{1a} and γ-aminobutyric acid (GABA). The effects of avermectin B_{1a} and GABA are both sensitive to inhibition by (+)-bicuculline. Avermectin B_{1a} and GABA both decrease the K_d and increase the B_{max} of [^{3}H]flunitrazepam binding to membranes. Kinetic analysis of the binding of [^{3}H]flunitrazepam to rat brain membranes indicates that avermectin B_{1a} and GABA reduce the rate constants of both association and dissociation between the ligand and the receptor. These results suggest a similar mechanism of modulation of benzodiazepine binding by avermectin B_{1a} and GABA. This modulation may involve an interaction among the receptors for benzodiazepine, GABA and avermectin B_{1a}.

Introduction

Avermectin B_{1a} *, a potent, wide-spectrum anthelmintic and insecticide [1,2], has been shown to mimic the action of γ-aminobutyric acid (GABA) in the lobster neuromuscular junction by opening Cl^- channels, thereby lowering membrane resistance [3]. This effect can be reversed by the Cl^- channel blocker picrotoxin [3]. Avermectin also blocks the signal transmission from ventral interneurons to excitatory motoneurons in the parasitic nematode *Ascaris* and this block is also reversible by picrotoxin [4]. In the rat brain, avermectin has been shown to increase specifically the release of [^{3}H]GABA from preloaded synaptosomes [5], to enhance benzodiazepine receptor binding in membranes and to potentiate the muscle relaxant effect of diazepam in mice [6].

The involvement of brain GABAnergic systems in mediating the muscle relaxant, anticonvulsant and anxiolytic effects of benzodiazepine in mammals has been demonstrated in pharmacological studies [7,8]. High affinity and stereospecific binding sites for benzodiazepine have been identified in rat brain membranes [9,10], and the selective stimulation of benzodiazepine receptor binding by GABA and GABA agonists has been reported [11,12]. These observations suggest an intimate relationship among avermectin, GABA and benzodiazepine actions on the GABAnergic system. We now present evidence indicating interactions among the avermectin, GABA and benzodiazepine recognition sites in the GABA postsynaptic receptor-Cl^- channel complex in rat brain membranes.

Materials and Methods

Materials. [^{3}H]Flunitrazepam (79.3 Ci/mmol) and [^{3}H]diazepam (79.9 Ci/mmol) were purchased from New England Nuclear. Unlabeled benzodiazepines were a gift from Hoffman-LaRoche, Inc. GABA, (+)-bicuculline and digitonin were obtained from Sigma. Avermectin B_{1a} was supplied by Merck Sharp & Dohme Research Laboratories.

Abbreviation: GABA, γ-aminobutyric acid.

* Throughout this paper avermectin B_{1a} is referred to simply as avermectin.

Preparation of rat brain synaptic membranes and solubilization of benzodiazepine receptors. Male Wistar rats (200–250 g each) were decapitated and the brains were immediately removed, freed of medulla and homogenized at 0°C in 15 vol. of 0.32 M sucrose, 0.5 mM $CaCl_2$, 1 mM $MgCl_2$ and 1 mM $NaHCO_3$ in a Teflon homogenizer. The homogenate was centrifuged at $1500 \times g$ for 10 min and the supernatant was recentrifuged at $17\,000 \times g$ for 10 min. The pellets were frozen at −20°C for 3 h or longer and resuspended in 10 mM sodium phosphate buffer, pH 7.4. After incubation at 0°C for 30 min the suspension was centrifuged at $25\,000 \times g$ for 30 min. This freezing-thawing-centrifugation step was repeated five times and the final pellets were used for benzodiazepine binding studies or for preparing solubilized benzodiazepine receptors. To solubilize the benzodiazepine receptor, the pellets were suspended at 5 mg protein/ml in 1% digitonin, 0.01 M sodium phosphate buffer, pH 7.4 and 0.32 M sucrose [13]. The mixture was stirred for 30 min at 0°C and centrifuged at $100\,000 \times g$ for 60 min. The supernatant fluid was used for benzodiazepine binding assays.

Benzodiazepine binding assays. Assays of benzodiazepine binding to brain membranes were performed in borosilicate glass disposable culture tubes (Fisher Scientific Co.) in 1 ml of 50 mM Tris-HCl buffer, pH 7.4 containing 60–100 μg protein, 0.5 nM [^{3}H]flunitrazepam, and the drug being examined in 1.0 μl dimethyl sulfoxide (DMSO). Incubation was carried out at 0°C for the time indicated. The incubated samples were filtered through Whatman GF/B filters and rinsed three times with 5.0 ml of the ice-cold Tris-HCl buffer. For assays of benzodiazepine binding to solubilized receptors, 200 μl of the $100\,000 \times g$ supernatant fraction was added to 0.3 ml of 50 mM Tris-HCl pH 7.4 containing 0.5 nM [^{3}H]flunitrazepam and the drug in 0.5 μl DMSO. The mixture was incubated at 0°C for 30 min and the protein precipitated with 0.5 ml of saturated ammonium sulfate solution. After another 3 min of incubation, the samples were filtered through GF/B filters and washed once with 7 ml of 50% saturated ammonium sulfate solution. The radioactivity on the filter was counted in Aquasol II (New England Nuclear) in a Beckman LS 8000 liquid scintillation counter. Specific [^{3}H]flunitrazepam binding was calculated by

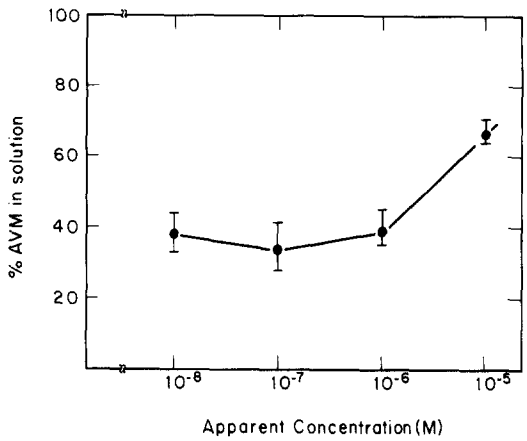

Fig. 1. The effective concentrations of avermectin (AVM) in solution. A 1 μl DMSO solution of [^{3}H]avermectin at various concentrations was mixed with 1 ml of 50 mM Tris-HCl buffer, pH 7.4 at 0°C. After 90 min, the percentage of radioactivity recovered from solution after a brief centrifugation was determined. Values shown are means of six experiments. The vertical bars are standard deviations.

subtracting the nonspecific binding (obtained in the presence of 5 μM clonazepam) from the total binding. Using [^{3}H]avermectin (3.1 Ci/mmol) as a tracer, the actual avermectin concentrations in the assay solution and 0.1% DMSO were corrected (Fig. 1). Most of the lost avermectin can be recovered from the test tube walls by washings with Aquasol 2.

For association kinetic studies, specific benzodiazepine binding to synaptic membranes (60 μg protein) at 0°C was measured at various time intervals after addition of 0.5 nM [^{3}H]flunitrazepam or 1 nM [^{3}H]diazepam. The association rate constant was calculated from the equation

$$k_{+1} = \frac{2.303}{t(L-R)} \log \frac{R(L-X)}{L(R-X)}$$

as described by Bennett [14], where L is the initial concentration of [^{3}H]flunitrazepam, R is the initial concentration of receptor, t is the incubation time, and X is the amount of ligand bound at the given time. To determine the dissociation rate constant, k_{-1}, synaptic membranes were incubated at 0°C with 0.5 nM [^{3}H]flunitrazepam for 120 min or 1 nM [^{3}H]-diazepam for 90 min to reach equilibrium and then excess unlabeled clonazepam (final concentration,

5 μM) was added. After various time intervals, specifically bound radioligand was measured. The half-life ($t_{1/2}$) for the loss of specifically bound radioligand was calculated from a plot of log(RL) versus time and k_{-1} was determined by multiplying the slope of the same plot by −2.303.

Results

Avermectin and GABA increase the binding of [^{3}H]flunitrazepam to washed membranes from rat brain in a dose dependent fashion with half maximal effective concentrations (EC_{50}) at 20 nM and 1.1 μM, respectively; and with near maximal effects at 7 μM for avermectin and 100 μM for GABA (Fig. 2). Avermectin is 20- to 50-fold more potent than GABA and the maximal stimulation by avermectin is approx. 200–300% greater than that by GABA. The combination of the two drugs at 100 μM GABA and 7 μM avermectin gave greater activation of [^{3}H]flunitrazepam binding than either avermectin or GABA alone (Table I). The GABA antagonist (+)-bicuculline [15] had a small effect on control [^{3}H]flunitrazepam binding but it inhibited GABA- and avermectin-potentiated binding by 95 and 56%, respectively. The dose-dependent inhibition of avermectin- and GABA-stimulated [^{3}H]flunitrazepam binding by (+)-bicuculline is shown in Fig. 3. Picrotoxin (100 μM), an inhibitor

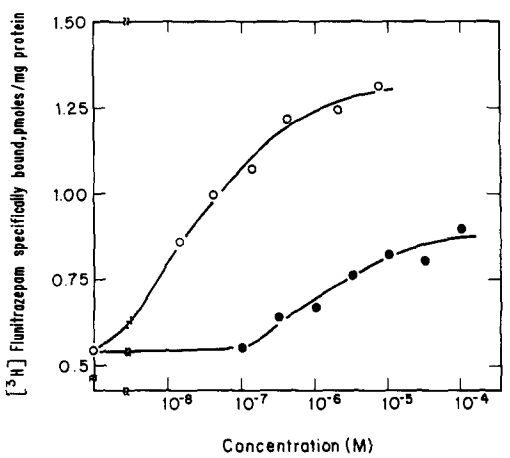

Fig. 2. The effect of varying concentrations of avermectin (AVM) and GABA on specific [^{3}H]flunitrazepam binding to rat brain synaptic membranes. o———o, AVM; •———•, GABA. The experiment was repeated three times with less than 20% variation.

TABLE I

EFFECT OF (+)-BICUCULLINE ON AVERMECTIN- AND GABA-STIMULATED [^{3}H]FLUNITRAZEPAM BINDING TO RAT BRAIN SYNAPTIC MEMBRANES

Treatment	[^{3}H]-Flunitrazepam specifically bound [a] (pmol/mg protein)	% Inhibition of stimulated benzodiazepine binding by (+)-bicuculline [b]
Control	0.64 ± 0.003	
+ 100 μM (+)-bicuculline	0.59 ± 0.02	–
7 μM avermectin (AVM)	1.16 ± 0.01 [c]	
+ 100 μM (+)-bicuculline	0.84 ± 0.01	56
100 μM GABA	0.88 ± 0.02	
+ 100 μM (+)-bicuculline	0.61 ± 0.02	95
7 μM AVM + 100 μM GABA	1.29 ± 0.02 [c]	
+ 100 μM (+)-bicuculline	0.99 ± 0.03	43

[a] Data are expressed as mean ± S.D.

[b] The values were calculated after subtracting (+)-bicuculline-insensitive control benzodiazepine binding (0.59 pmol/mg protein).

[c] Significantly different from each other ($P < 0.01$).

of the GABA-mediated chloride ion channel [16], had no effect on control, avermectin- or GABA-stimulated binding (data not shown). The effects of chloride ion on the activation of [^{3}H]flunitrazepam binding by avermectin and GABA were studied. The addition of NaCl (100 mM) to an assay buffer of 50 mM Tris acetate produced a small increase in control binding but caused a substantial increase in the binding already activated by avermectin or GABA (Table II). When acetate ion was added to substitute for chloride ion in the assay so that the ionic strength was constant, the concentration of chloride ion needed for maximal avermectin activation of benzodiazepine binding was 30–40 milliequivalents (Fig. 4). However, no significant effect of chloride ion was observed on the GABA-stimulated benzodiazepine binding. The increase in the GABA activation by 100 mM NaCl noted in Table II thus could be caused by an increase in ionic strength.

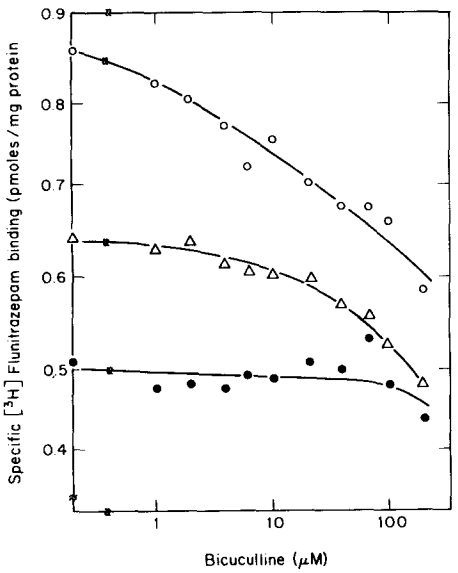

Fig. 3. Effect of (+)-bicuculline on avermectin (AVM)- and GABA-activated and control [^{3}H]flunitrazepam binding to rat brain synaptic membranes. ●——●, control; ○——○, 7 μM AVM and △——△, 100 μM GABA. Each point represents an average of triplicates and the experiment was repeated three times with less than 10% variation.

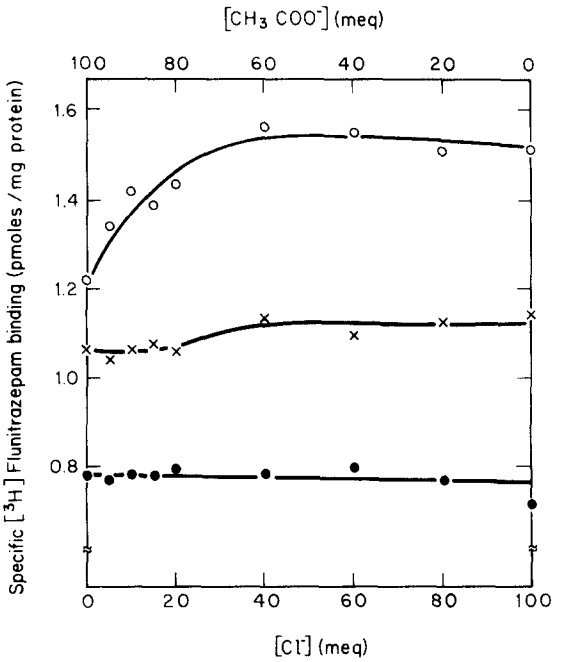

Fig. 4. Effect of Cl^- concentration on avermectin (AVM)- and GABA-activated and control [^{3}H]flunitrazepam binding to rat brain synaptic membrane. The buffer used was prepared by mixing 100 mM Tris-HCl, pH 7.4 and 100 mM Tris acetate, pH 7.4 in varying proportions to maintain a constant ionic strength in all experiments. ●——●, control; ○——○, 7 μM AVM and X——X, 100 μM GABA. Each data point represents an average of triplicate samples and the experiment was repeated three times with less than 10% variation.

To determine if avermectin and GABA potentiate the binding of [^{3}H]flunitrazepam to solubilized receptors, the synaptic membranes were treated with 1% digitonin. The yield of solubilization for benzodiazepine receptor was approx. 42% and the non-specific binding was 15 to 25% of the total binding. Avermectin and GABA increased the specific binding of [^{3}H]flunitrazepam to solubilized receptors by 39 and 36%, respectively (Table III). (+)-Bicuculline (100 μM) inhibited GABA-activated and avermectin-activated binding by 58 and 61%, respectively.

TABLE II

EFFECT OF NaCl ON AVERMECTIN- AND GABA-STIMULATED [^{3}H]FLUNITRAZEPAM BINDING TO RAT BRAIN SYNAPTIC MEMBRANES

Treatment	Percent stimulation of [^{3}H]flunitrazepam binding, mean ± S.D.
+ 100 mM NaCl	1.2 ± 0.4
+ 7 μM avermectin (AVM)	64.1 ± 2.1
+ 7 μM AVM + 100 mM NaCl	116.2 ± 3.4
+ 100 μM GABA	29.2 ± 1.6
+ 100 μM GABA + 100 mM NaCl	51.1 ± 1.5

Scatchard analysis of specific [^{3}H]flunitrazepam binding to synaptic membranes indicates that the avermectin-stimulated binding is due to a change in the affinity of the receptor for ligand as well as a change in the number of detectable binding sites (Fig. 5). In control samples, the dissociation constant (K_d) for [^{3}H]flunitrazepam was 1.01 ± 0.11 nM and the density of binding sites (B_{max}) was 2.79 ± 0.17 pmol/mg protein. In the presence of 7 μM avermectin, the K_d was decreased to 0.55 ± 0.08 nM and the B_{max} was increased to 4.04 ± 0.41 pmol/mg protein. In the presence of 100 μM GABA, the K_d and B_{max} were 0.57 ± 0.11 nM and 3.19 ± 0.34 pmol/mg protein, respectively. The Hill coefficients were 1.01,

TABLE III

STIMULATION BY AVERMECTIN AND GABA AND INHIBITION BY (+)-BICUCULLINE OF [^{3}H]FLUNITRAZEPAM BINDING TO DIGITONIN-SOLUBILIZED BENZODIAZEPINE RECEPTOR COMPLEX FROM RAT BRAIN

Treatment	[^{3}H]-Flunitrazepam specifically bound [a] (pmol/mg protein)	% Inhibition (+)-bicuculline of stimulated benzodiazepine binding
Control	0.64 ± 0.04	
+ 100 μM (+)-bicuculline	0.58 ± 0.04	–
7 μM avermectin (AVM)	0.89 ± 0.03 [c]	
+ 100 μM (+)-bicuculline	0.70 ± 0.08 [d]	61 [b]
100 μM GABA	0.84 ± 0.04 [c]	
+ 100 μM (+)-bicuculline	0.69 ± 0.03 [d]	58 [b]

[a] Data are expressed as mean ± S.D. The experiments were repeated three times in separate preparations of solubilized brain membranes.

[b] These values were calculated after subtracting (+)-bicuculline-insensitive control benzodiazepine binding.

[c] Significantly different from control ($P < 0.005$).

[d] Significantly different from the experiment without (+)-bicuculline ($P < 0.01$).

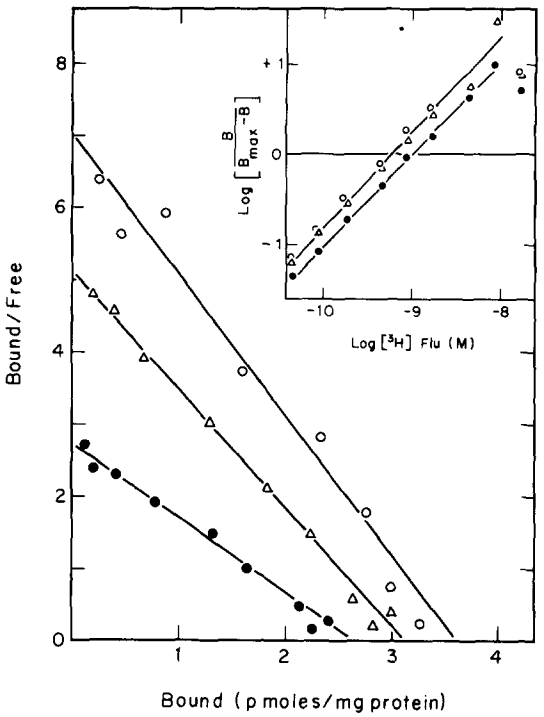

Fig. 5. Scatchard analysis of avermectin (AVM)- and GABA-activated and control [^{3}H]flunitrazepam binding to rat brain synaptic membranes. (●———●), control; ○———○, 7 μM AVM; and △———△, 100 μM GABA. The experiment was repeated three times and the values obtained varied by less than 15%.

TABLE IV

RATE CONSTANTS OF ASSOCIATION AND FAST PHASE DISSOCIATION FOR BENZODIAZEPINE BINDING IN THE PRESENCE OF AVERMECTIN (AVM) AND GABA

These values are means of five separate experiments. The concentrations of [^{3}H]flunitrazepam and [^{3}H]diazepam were 0.5 nM and 1.0 nM, respectively.

	$k_{+1} \times 10^{-7}$ ($M^{-1} \cdot min^{-1}$)	k_{-1} (min^{-1})	k_{-1}/k_{+1} (nM)	K_d [a] (nM)
(1) [^{3}H]Flunitrazepam				
Control	5.04 ± 0.55	0.0746 ± 0.0019	1.48	1.01
AVM (7 μM)	3.65 ± 0.38	0.0234 ± 0.0047	0.64	0.55
GABA (100 μM)	4.12 ± 0.62	0.0503 ± 0.003	1.22	0.57
(2) [^{3}H]Diazepam				
Control	4.62 ± 0.16	0.361 ± 0.047	7.8	–
AVM (7 μM)	4.32 ± 0.32	0.207 ± 0.056	4.8	–
GABA (100 μM)	5.30 ± 0.37	0.238 ± 0.031	4.5	–

[a] K_d was calculated from experiments described in Fig. 5.

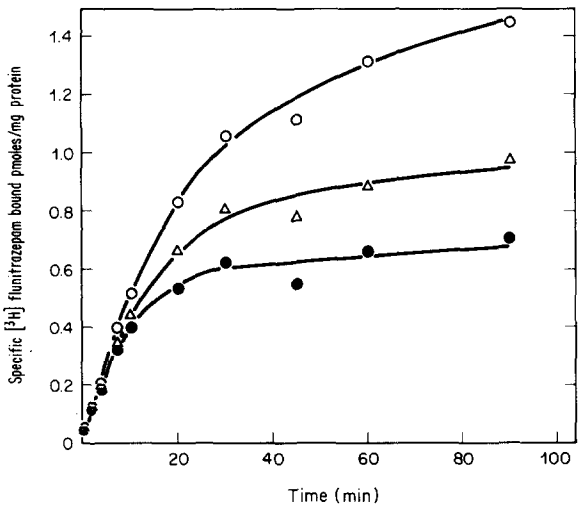

Fig. 6. Effect of avermectin (AVM) and GABA on the association rate of [^{3}H]flunitrazepam binding to rat brain synaptic membranes. Specific benzodiazepine binding to synaptic membranes (60 μg protein) at 0°C at various time intervals after the addition of 0.5 nM [^{3}H]flunitrazepam was assayed. •, control; ○, 7 μM AVM and △, 100 μM GABA. The experiment was repeated five times with less than 20% variation in the association rate constants as shown in Table IV.

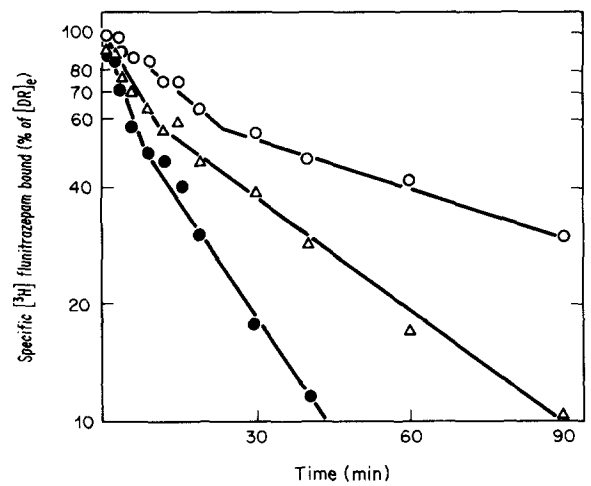

Fig. 7. The dissociation kinetics of [^{3}H]flunitrazepam binding to rat brain synaptic membranes in control samples (•) or in samples containing either 100 μM GABA (△) or 7 μM avermectin (○). The term $[DR]_e$ on the ordinate is the amount of [^{3}H]flunitrazepam bound at equilibrium. The experiment was repeated five times with less than 20% variation in the dissociation rate constants as shown in Table IV.

1.06 and 1.07 for control, GABA- and avermectin-activated [^{3}H]flunitrazepam binding, respectively (see Fig. 5 inset).

The rate constants of association and dissociation between [^{3}H]flunitrazepam and the receptor in the synaptic membrane in the presence or absence of avermectin and GABA were determined (Table IV). In control samples, the association between the radioactive ligand and the receptor reached equilibrium within 20–30 min, while the avermectin-activated [^{3}H]flunitrazepam binding required 90–120 min to achieve equilibrium (Fig. 6). Similarly, GABA also decreased the association rate constant (Fig. 6 and Table IV). The dissociation of radioligand from the receptor in control samples proceeded in two phases; a fast phase which had a $T_{1/2}$ value of 8.5 ± 1.1 min and a slow phase which had a $T_{1/2}$ of 15.1 ± 1.2 min (Fig. 7). In the presence of avermectin the dissociation also had two phases but the rates were much slower; the $T_{1/2}$ values were 30.5 ± 5.6 and 60.6 ± 10.9 min, respectively. Practically all the bound [^{3}H]flunitrazepam was dissociated from receptors within 1 h in control samples, but it required 4 h for the dissociation to reach completion when avermectin was present. In the presence of GABA, a similar decrease in the dissociation rate was observed; the $T_{1/2}$ values for the fast and slow phases were 13.2 ± 0.5 and 32.6 ± 3.4 min, respectively (Fig. 7). These data were used to calculate the rate constants of association (k_{+1}) and dissociation (k_{-1}) by assuming a second-order reaction for association and a first-order reaction for dissociation. The results in Table IV indicate that both the k_{+1} and the fast phase k_{-1} values for [^{3}H]flunitrazepam binding are reduced significantly by avermectin or GABA. Similar results were obtained when [^{3}H]diazepam was used as the radioligand, but only the fast phase k_{-1} values were significantly lowered by avermectin and GABA (Table IV). The dissociation constants calculated from k_{-1}/k_{+1} are close to the K_d values estimated from Scatchard analysis of the binding data of both radioligands obtained at equilibrium in separate experiments (Fig. 5).

Discussion

Several lines of evidence suggest that there are distinct avermectin, GABA and benzodiazepine binding sites which interact within the same receptor complex

in the rat brain synaptic membrane, most likely the GABA postsynaptic receptor-chloride ion channel complex. Both avermectin and GABA increase benzodiazepine binding to synaptic membranes and, more importantly, to digitonin-solubilized receptors. The dose-dependent inhibition by the GABA antagonist (+)-bicuculline of avermectin- as well as GABA-stimulated benzodiazepine binding to brain membranes and solubilized benzodiazepine receptors suggests that the avermectin activation of benzodiazepine receptors involves a GABA-mediated mechanism and that an unhindered GABA recognition site may be essential for the stimulatory action of avermectin on benzodiazepine receptor binding. It thus appears that the avermectin binding site may be physically linked to the GABA recognition site. Experiments involving the protection of benzodiazepine and GABA receptors from inactivation by heat and iodoacetamide have led to a similar conclusion that GABA and benzodiazepine sites are allosterically coupled [17]. The maximal effect of avermectin on benzodiazepine receptor binding in brain membrane preparations is greater than that of GABA and the combination of both drugs gives greater, though not additive, stimulation than either alone suggesting that the mechanisms of the modulatory effects of avermectin and GABA may be similar but not identical. We have previously shown that specific avermectin binding is not inhibited by either GABA or diazepam [18].

The experimental results on the stimulatory effect of Cl^- on avermectin-activated benzodiazepine-receptor binding agree with the general concept that the benzodiazepine receptor is coupled to a chloride ion channel [12,19]. However, since Cl^- has no effect on GABA-potentiated benzodiazepine binding, the Cl^- dependence of the avermectin effect may be primarily due to a close relationship between the avermectin-binding site and a Cl^- channel. This hypothesis is supported by our recent findings of a stimulatory effect of avermectin on GABA binding that is blocked by picrotoxin and required the presence of chloride ion [20]. In the present study, picrotoxin was found to be inactive in blocking the effects of avermectin and GABA on benzodiazepine-receptor binding, it is therefore unlikely that avermectin and picrotoxin share the same binding site. These data, taken together with the Cl^- involvement in the action of avermectin on the lobster neuromuscular junction [3] and in GABA function in mammalian brain [19,20], support the hypothesis that avermectin acts on a modulatory site which is distinct from the GABA or benzodiazepine recognition sites in the GABA postsynaptic receptor-chloride ion channel complex.

Other investigators have also reported stimulation of benzodiazepine binding by GABA in fractions of brain membranes obtained after solubilization by Triton X-100 or sodium deoxycholate [21,22]. The stimulation by avermectin and GABA of benzodiazepine binding to a digitonin solubilized fraction reported here suggests that the same macromolecule or complex possesses the recognition sites for avermectin, GABA and benzodiazepine. However, two or more distinct receptor proteins in the soluble state can still interact. Work is in progress to purify the receptor(s) in order to further clarify the nature of these interactions.

Scatchard analysis shows that avermectin increases the number of detectable benzodiazepine-binding sites (B_{max}) and their affinity (K_d) which agrees with similar observations using [^{3}H]diazepam as the radioligand [6,23]. In the case of potentiation of benzodiazepine binding by GABA, it was first attributed primarily to an increase in the affinity of benzodiazepine for its receptor [11], but a change of B_{max} has also been noted recently [24]. We found GABA, in addition to increasing the affinity of benzodiazepine binding, also caused a small, but statistically significant, increase in B_{max}. Multiple receptors for benzodiazepine have been documented [25,26] and it has been postulated that only one of these is physically linked to the GABA receptor. It is unclear whether the benzodiazepine receptor affected by avermectin is different from that seen in the absence of avermectin, or whether the same receptor undergoes a configuration change when avermectin is present, resulting in a higher affinity for benzodiazepine. It is also possible that avermectin may act to remove an endogenous inhibitor of benzodiazepine or GABA binding [27].

Kinetic analysis indicates that both the association and dissociation components of avermectin- and GABA-activated benzodiazepine binding to synaptic membranes are slower than the control. The K_d values determined from the ratios of the dissociation rate constants to the association rate constants are close to those independently obtained from Scatchard analysis. In both cases, the decrease in K_d can

be attributed to a more extensive decrease in the dissociation rate constant. The conincidence of the action of averm ctin and GABA in this aspect indicates that a similar mechanism of action may be involved. A similar effect of GABA on the association and dissociation of benzodiazepine-receptor binding has been reported [28], but differs from an earlier study using [^{3}H]diazepam as radioligand [11]. Using [^{3}H]diazepam we did not find a significant change in the association rate constant but found a significant decrease in the dissociation rate constant in the presence of GABA or avermectin.

Although no benzodiazepine receptors have yet been found in invertebrates [29], avermectin is known to increase picrotoxin-sensitive chloride ion permeability in the lobster neuromuscular junction [3] which suggests an avermectin effect on GABA-mediated neurotransmission. The present data indicate that there may be an interaction between GABA and avermectin-recognition sites in addition to their interaction with benzodiazepine-recognition sites in rat brain. Thus it is possible that during the evolution of the GABA receptor complex the avermectin-recognition site was acquired earlier than the benzodiazepine-recognition site.

In conclusion, our data indicate that avermectin and GABA act by similar mechanisms to modulate benzodiazepine receptor binding. Thus avermectin, GABA and benzodiazepine may interact allosterically at distinct sites on the GABA postsynaptic receptor-Cl^- ion-channel complex. Acermectin has also been shown to cause an increase in the release of GABA from rat brain synaptosomes [5]. These two mechanisms of action of avermectin, both leading to the potentiation of GABA-mediated transmission, may account for its muscle relaxant effect in rats [6] and its inhibition of neurotransmission in the lobster neuromuscular junction [3] and the parasitic nematode *Ascaris* [4].

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