Displacement of DL-[^{3}H]-2-Amino-4-phosphonobutanoic Acid ([^{3}H]APB) Binding with Methyl-Substituted APB Analogues and Glutamate Agonists†

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ABSTRACT: The binding of the excitatory amino acid antagonist DL-2-amino-4-phosphonobutanoic acid (DL-APB) to rat brain synaptic plasma membranes was characterized. As determined by Scatchard analysis, the binding was saturable and homogeneous with a K_d = 6.0 μM and B_{max} = 380 pmol/mg of protein. The binding was dependent on the presence of Ca^{2+} and Cl^- ions and was diminished upon freezing. The association rate constant was 6.8 × 10^{-3} μM^{-1} min^{-1}, and the dissociation rate constant was 2.0 × 10^{-2} min^{-1}. The L isomers of APB, glutamate, and aspartate were more potent as displacers of APB binding than the D isomers. Previously determined inhibition data obtained for APB-sensitive inputs to hippocampal granule cells are compared to the present displacement data in an attempt to identify this binding protein as the recognition site of the receptor mediating the APB-induced inhibition of synaptic transmission. With the exception of kynurenic acid, all compounds examined in both systems were more potent as displacers of APB binding than as inhibitors of synaptic transmission. This difference in potency was most pronounced for agonists at dentate granule cells. L-Glutamate, D-glutamate, and L-glutamate tetrazole were between 140- and 7500-fold more potent as displacers of DL-APB binding than as inhibitors of synaptic transmission. D-2-Amino-5-phosphonopentanoic acid and α-methyl-APB were between 10- and 20-fold more potent as displacers of binding. Three monomethyl-substituted APB analogues (with methyl groups incorporated at the phosphonate, β, and γ positions) were between 25- and 30-fold more potent as displacers of APB binding than as antagonists of evoked responses in the lateral perforant path, while L-APB is equipotent in both assays. The parallel increases in potency of the methyl-substituted derivatives are the only observation that suggests this binding protein may be a modified form of the receptor mediating the inhibition of lateral perforant path responses by L-APB. On the other hand, the lack of a similar increase for L-APB itself, the significantly altered kinetics of association and dissociation, and the lack of correlation between the ligand specificity of this binding site and the currently accepted pharmacology of "glutamate" receptors do not support this hypothesis.

At presumed glutamatergic pathways (Fagg & Foster, 1983), the glutamate analogue L-2-amino-4-phosphonobutanoic acid (L-APB)[1] is a potent and specific antagonist (apparent K_d = 1–10 μM). APB inhibits synaptic transmission, with the L isomer approximately 20-fold more potent than the D isomer in the following pathways: the lateral entorhinal projections to the hippocampal granule cells (the lateral perforant path) (Koerner & Cotman, 1981), the lateral olfactory tract projections to the pyramidal cells in the prepyriform cortex (Collins, 1982; Hori et al., 1982), projections from hippocampal granule cells to the CA3 pyramidal cells (Robinson et al., 1984a; Yamamoto et al., 1983), and projections to the dorsal horn neurons in the spinal cord (Davies & Watkins, 1982; Evans et al., 1982).

One class of L-[^{3}H]glutamate binding sites is displaceable by DL-APB with IC_{50}'s in the 1–10 μM range (Baudry & Lynch, 1981; Fagg et al., 1982; Larder & McLennan, 1982; Werling et al., 1983). The L isomer of APB is a more potent displacer of this binding than is the D isomer. These observations led to the suggestion that this binding site might be the recognition site of the receptor mediating the APB-induced antagonism of synaptic responses (Fagg et al., 1982). The recent availability of tritium-labeled DL-APB has allowed examination of a binding site with similar ligand specificity (Butcher et al., 1983; Monaghan et al., 1983). Under the conditions used in such studies, the binding appears homogeneous and is competitively displaced by L-glutamate. It is thus possible to compare the ligand specificity of a single glutamate-sensitive binding site to pharmacological data.

We have recently synthesized a series of methyl-substituted APB analogues and examined the potency of these compounds in the lateral perforant path (Freund et al., 1984; Crooks et al., 1985), where L-APB is most potent (Koerner & Cotman, 1981). These analogues have a broad range of potencies in this pathway. The purpose of this investigation was to compare the ligand selectively of the previously described APB binding site to the pharmacology of several APB analogues in the lateral perforant pathway. We report here that for the antagonists the rank order of the IC_{50}'s as displacers of DL-[^{3}H]APB binding parallels the potency of these analogues in the lateral perforant pathway, but the increases in potency are parallel only for the APB derivatives and cannot be extended

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[1] Abbreviations: APB, 2-amino-4-phosphonobutanoic acid; D-APV, D-2-amino-5-phosphonopentanoic acid; L-glutamate tetrazole, L-2-amino-4-(5-tetrazolyl)butanoic acid; DL-AMPB, DL-2-amino-4-(methylphosphino)butanoic acid; α-methyl-APB, 2(*RS*)-amino-2-methyl-4-phosphonobutanoic acid; β-methyl-APB, 2(*RS*)-amino-3(*RS*)-methyl-4-phosphonobutanoic acid; γ-methyl-APB, 2(*RS*)-amino-4(*RS*)-phosphonopentanoic acid; IC_{50}, concentration inhibiting 50% of the activity; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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to L-APB. A preliminary account of these results has been presented (Robinson et al., 1984b).

Experimental Procedures

Materials

DL-[3,4-^{3}H]-2-Amino-4-phosphonobutanoic acid (26.1 Ci/mmol) was obtained from New England Nuclear. DL-2-Amino-4-phosphonobutanoic acid was obtained from Calbiochem. Kynurenic acid, kainic acid, L- and D-glutamic acids, L- and D-aspartic acids, and HEPES acid were obtained from Sigma Chemical Co. D-2-Amino-5-phosphonopentanoic acid, L-2-amino-4-phosphonobutanoic acid, and L-quisqualic acid were obtained from Cambridge Research Biochemicals. L-2-Amino-4-(5-tetrazolyl)butanoic acid (Koerner et al., 1983), DL-2-amino-4-(methylphosphino)butanoic acid (Freund et al., 1984), 2(*RS*)-amino-2-methyl-4-phosphonobutanoic acid, 2(*RS*)-amino-3(*RS*)-methyl-4-phosphonobutanoic acid, and 2(*RS*)-amino-4(*RS*)-phosphonopentanoic acid (Crooks et al., 1985) were prepared as previously described.

Methods

Preparation of Synaptic Plasma Membranes. Our preparation of synaptic plasma membranes was based on modifications (Foster et al., 1981a) of the procedure developed by Cotman & Taylor (1972). Except where noted, centrifugations were performed in a Ti 50.2 fixed angle rotor (Beckman Instruments), and the preparation was kept at 4 °C. Holtzman rats, 30–60 days old, were sacrificed by cervical dislocation; the forebrain was removed and homogenized (600 rpm, 7 strokes) in a 30% w/v (dilutions refer to wet weight) solution containing 0.2 mM HEPES–KOH buffer (pH 7.4), 50 μM $CaCl_2$, and 0.32 M sucrose (solution A). This homogenate was diluted to 10% w/v with solution A and centrifuged at 1500*g* for 4 min. The pellets were resuspended in 20% w/v solution A, rehomogenized, and centrifuged as performed above. The supernatants were combined and centrifuged at 17000*g* for 8 min. To lyse this membrane fraction, the pellets were resuspended in 10% w/v 0.2 mM HEPES–KOH buffer (pH 7.4) containing 50 μM $CaCl_2$ (solution B) and homogenized (300 rpm, 4 strokes; all subsequent homogenizations were performed in this manner). This mixture was incubated at 4 °C for 15 min and rehomogenized. Potassium phosphate buffer (pH 7.4) containing *p*-iodonitrotetrazolium violet and sodium succinate was added to produce final concentrations of 40, 1, and 15 mM; respectively. This mixture was incubated 20 min at 33 °C and then centrifuged at 50000*g* for 25 min. The resulting pellet was resuspended (to 3 mL/g wet weight) by homogenization in solution B containing 0.85 M sucrose, layered on a discontinuous density gradient composed of 0.85, 1.0, and 1.3 M sucrose, and centrifuged in an SW 28 rotor at 70000*g* for 100 min. The synaptic plasma membranes were isolated at the 1.0–1.3 M interface. To remove endogenous inhibitors of binding, the membranes were suspended in solution B and centrifuged at 55000*g* for 20 min. After each wash, the membranes were rehomogenized in solution B and diluted 100-fold. The membranes were washed a total of 4 times. The final pellet was suspended in solution B to a final concentration of 3–5 mg of protein/mL as determined by the Lowry protein assay using bovine serum albumin as a standard (Lowry et al., 1951).

Centrifugation Binding Assays. Binding assays were performed in 50 mM HEPES–KOH buffer (pH 7.4), 10 mM $CaCl_2$ (20 mM Cl^-), and no more than 200 μg of protein/mL in a total volume of 1 mL. Immediately before use, the membranes were diluted in the above solution and preincubated at 33 °C for 5 min. Assays were initiated by the addition of ligand and displacer in a volume of 200 μL. The assay mixtures were vortexed vigorously and then incubated for 1 h at 33 °C in a shaker water bath. To prevent excessive aggregation of the membranes, the tubes were inverted every 15 min. The assays were terminated by centrifugation at 12500*g* in a microfuge (Brinkman Instruments, Inc.) for 3 min. The supernatant was aspirated and the top of the pellet rapidly washed by briefly layering cold buffer over the pellet. The pellets were solubilized in sodium dodecyl sulfate (1 mL) overnight and diluted with 8 mL of Aquasol 2 (New England Nuclear), and the bound radioactivity was counted by liquid scintillation spectrometry at a counting efficiency of approximately 40%. Assays were performed in triplicate, and the nonspecific contribution to binding was determined from parallel assays containing excess unlabeled ligand (1 mM). The levels of nonspecific binding were between 13 and 60% of the total bound, depending on the concentration of ligand used. Less than 5% of the ligand was bound at any ligand concentration; for this reason the free ligand concentration was assumed to be the total ligand concentration.

Data for concentration–percent inhibition curves were obtained from triplicate assays on at least three different membrane preparations. The reported IC_{50}'s are weighted means of IC_{50}'s calculated for each experimental point. The weighting factor assigned to each point was proportional to the square of the slope of a theoretical log concentration–percent inhibition curve at its intersection with that point.

Results

DL-[^{3}H]APB binding was linear with increasing protein concentration to approximately 250 μg/mL. At greater concentrations, the amount of binding began to saturate with respect to protein concentration. In one experiment, data for a Scatchard analysis were taken immediately after preparation of the synaptic plasma membranes (K_d = 4.2 μM and B_{max} = 420 pmol/mg of protein) and then again 42 h later (K_d = 6.9 μM and B_{max} = 260 pmol/mg of protein). These results are in agreement with previous results showing that binding activity decreases with time (Foster et al., 1981a). To reduce variation, saturation and kinetic analyses were performed between 14 and 18 h after preparation of the membranes. A Scatchard analysis (Figure 1) was performed, and the data were fit by linear regression analysis (K_d = 6.0 μM and B_{max} = 380 pmol/mg of protein). The time course for association of ligand and binding protein (Figure 2) demonstrates that it takes approximately 50 min for the binding to come to equilibrium. With the data from this association time course, a linear plot, which assumes pseudo-first-order kinetics for a bimolecular reaction, was performed by the method of Weiland & Molinoff (1981) (eq 5). The slope of this line and the binding site concentration, determined from the Scatchard analysis, were used to calculate the association rate constant ($k_1 = 6.8 \times 10^{-3}$ μM^{-1} min^{-1}). The dissociation rate constant was determined by incubating the membranes in the presence of ligand for 1 h followed by the addition of excess DL-APB (1 mM). The first-order dissociation plot (Figure 3) was fit by linear regression analysis [slope ($-k_{-1}$) = -2.0×10^{-2} min^{-1}], corresponding to a $t_{1/2}$ of 35 min. The dissociation constant calculated from the two kinetic constants was 2.9 μM. The linear plot for the dissociation experiments did not intercept with the expected zero time point, suggesting that 10% of the binding sites may have a substantially larger dissociation rate constant.

The previously described APB-displaceable L-[^{3}H]glutamate binding activity is dependent on the presence of Ca^{2+}/Cl^- ions (Fagg et al., 1982) and abolished upon freezing (Fagg et al.,

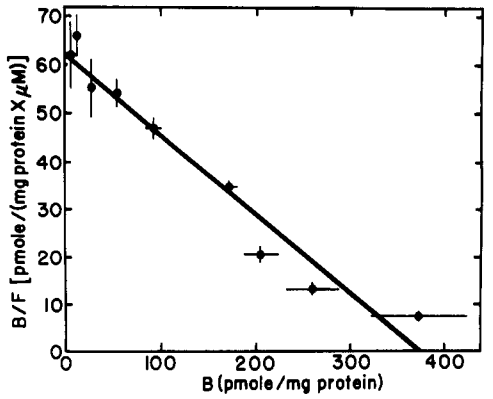

FIGURE 1: Scatchard analysis of DL-[^{3}H]APB binding. Synaptic plasma membranes were incubated with DL-[^{3}H]APB (0.10–50 μM) for 1 h. *B* represents the amount of ligand bound at the concentration *F* of ligand. Assays were performed 14–18 h after preparation of the synaptic plasma membranes. The dissociation constant and maximum level of binding were determined by linear regression analysis. Each point is the mean level of binding ± SEM determined from triplicate assays on four different membrane preparations.

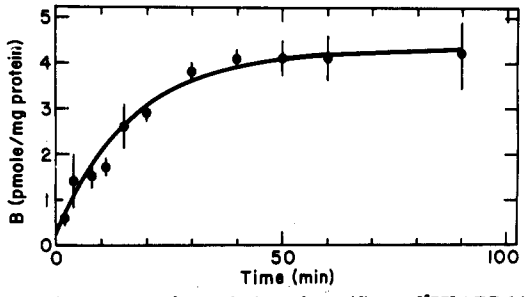

FIGURE 2: Time course of association of specific DL-[^{3}H]APB binding to synaptic plasma membranes. Synaptic membranes were incubated with DL-[^{3}H]APB (100 nM) for various times, and aliquots were removed and centrifuged. *B* denotes the amount of ligand bound. Each point is the mean level of binding ± SEM of triplicate assays on at least three different membrane preparations.

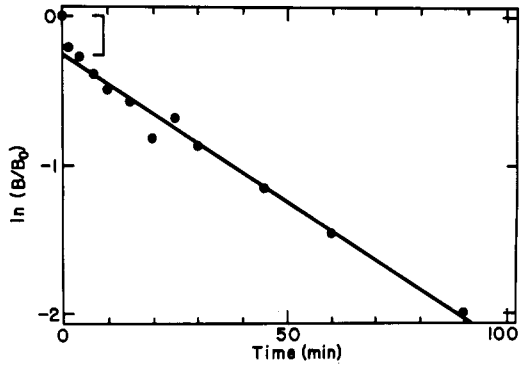

FIGURE 3: First-order dissociation plot of specific DL-APB binding from synaptic plasma membranes. Synaptic plasma membranes were incubated with DL-[^{3}H]APB (100 nM) for 1 h, the specific activity of the APB was infinitely diluted with excess unlabeled DL-APB (1 mM), and aliquots were removed at various times and centrifuged. *B* denotes the amount of ligand bound, and B_0 is the amount bound prior to addition of excess unlabeled ligand. Each point is the mean level of binding ± SEM of triplicate assays on four different membrane preparations. The plot was fit by linear regression analysis ignoring the zero time point and the dissociation rate constant determined from the slope of the line. The bracket indicates a rapidly dissociating component and the time needed to stop the reaction.

1983). To identify this APB binding site as the previously described glutamate binding site, these two properties were examined. The binding to membranes frozen at −20 °C for 1 week was reduced to less than 20% of the binding to fresh membranes. Substitution of the $CaCl_2$ with an equal concentration of $Mg(CH_3COO)_2$ or $MgCl_2$ resulted in 90% and 50% reductions, respectively, of the observed binding. These two observations support the hypothesis that the APB binding site described here corresponds to the previously described glutamate binding site.

Table I: Comparison of Displacement of DL-[^{3}H]APB Binding from Synaptic Plasma Membranes with the Potency of These Compounds in the Lateral Perforant Path[a]

	displacement of APB binding (D)		inhibition of field potentials recorded in the lateral perforant path (LPP)		ratio
compound	IC_{50} (μM)	n_{Hill}	IC_{50} (μM)	ag/ant	LPP/D
L-quisqualate	0.26[b]	1.0	ND	ag	
L-glutamate tetrazole	1.4	0.8	200[c]	ag	140
L-glutamate	1.6	1.0	12000[c]	ag	7500
L-aspartate	8.0	1.0	ND	ag	
D-glutamate	9.6	1.0	7500[c]	ag	780
D-aspartate	25	1.0	ND	ag	
L-APB	1.5	0.8	2.5[d]	ant	1.7
DL-AMPB	3.8	0.9	110[e]	ant	29
γ-methyl-APB	8.6	0.8	220[f]	ant	26
D-APV	15	1.0	300 (DL)[d]	ant	20
β-methyl-APB	18	0.9	500[f]	ant	28
D-APB	24	0.8	100[d]	ND	4.2

[a] The log concentration–percent inhibition curves were analyzed as described under Methods. Under these conditions, if the binding is competitive, the K_i's = $0.98IC_{50}$. Inhibition of lateral perforant path responses are previously reported values (see individual references; ND, not determined; ag, agonist; ant, antagonist). The ratio, LPP/D, is the ratio of the IC_{50} for inhibition in the lateral perforant path to IC_{50} for displacement of DL-APB binding. [b] The IC_{50} for quisqualate was calculated by assuming a 15% quisqualate-insensitive component. [c] Koerner et al. (1983). [d] Koerner & Cotman (1981). [e] Freund et al. (1984). [f] Crooks et al. (1985).

Table II: Comparison of Displacement of DL-[^{3}H]APB Binding by Excitatory Amino Acid Analogues That Displaced Less Than 50% of the Binding at 1 mM with the Potency of These Compounds in the Lateral Perforant Path[a]

	% inhibition at 1 mM, mean ± SEM	inhibition of field potentials recorded in the lateral perforant path	
compound	(*n*)	IC_{50} (μM)	ag/ant
kynurenate	22 ± 3 (3)	130[b]	ant
baclofen	34 ± 3 (3)	inactive[c]	
α-methyl-APB	39 ± 5 (3)	>10000[d]	
kainate	21 ± 3 (3)	ND	ag

[a] Synaptic plasma membranes were incubated with DL-[^{3}H]APB (100 nM) and displacer (1.0 mM) for 1 h followed by centrifugation. The level of inhibition is the mean ± SEM of triplicate assays on at least three different membrane preparations. Inhibition values of lateral perforant path responses are previously reported values (see individual references; ND, not determined; ag, agonist; ant, antagonist). [b] Robinson et al. (1984a). [c] Lanthorn & Cotman (1981). [d] Crooks et al. (1985).

The ligand specificity of this binding site was examined with both glutamate and APB analogues. Membranes were incubated in the presence of DL-[^{3}H]APB (100 nM) and displacer (3 nM–1 mM) as described under Methods. The level of binding in the absence of displacer was determined in parallel assays. Except for quisqualate, the data closely paralleled theoretical curves with Hill coefficients close to 1 (0.8–1.0) and approached 100% displacement at concentrations 10–100 times the IC_{50}.[2] The quisqualate displacement data were fit

[2] These data and theoretical curves were scrutinized by the reviewers and are available from the authors upon request.

to a theoretical curve that assumed a 15% quisqualate-insensitive component. The IC_{50}'s and Hill coefficients are summarized in Table I. Previously determined data for inhibition of synaptic responses of the lateral perforant path in hippocampal slices are also presented in Table I. Compounds displacing less than 50% of the binding at 1 mM were examined at a single concentration (1 mM). These results are presented in Table II. From the IC_{50}'s for D- and L-APB it was calculated that 90% of the binding was due to the L isomer at this concentration of ligand (100 nM). In agreement with the two previous reports describing the ligand specifity of a DL-[^{3}H]APB binding site (Butcher et al., 1983; Monaghan et al., 1983), this binding was most sensitive to quisqualate, was more sensitive to the L isomers of glutamate, aspartate, and APB than the D-isomers of these amino acids, and was weakly inhibited by kainate. All of the previously reported IC_{50}'s for binding are within a factor of 3, and most are within a factor of 2 compared with those presented in Table I. Displacement data presented here also agree with the previously described Ca^{2+}/Cl^- dependent glutamate binding site [see Foster & Fagg (1984)].

Receptors are initially biochemically identified by comparing known pharmacology to ligand specificity of a binding site. Thus, we have synthesized or obtained from commercial sources several APB analogues and have tested these analogues as inhibitors of lateral perforant path responses to identify the structural requirements necessary to inhibit this L-APB-sensitive pathway. These compounds possess a broad range of potencies at these synapses. L-Glutamate tetrazole, an agonist, was the second most potent displacer of DL-[^{3}H]APB binding with an IC_{50} = 1.4 μM (Table I). DL-AMPB, and β-methyl-APB had IC_{50}'s between 3.8 and 18 μM (Table I). γ-Methyl-APB was much weaker, displacing 39% of the binding at 1 mM (Table II). Baclofen [(−)-β-(*p*-chlorophenyl)-γ-aminobutanoic acid] and kynurenate were also weak displacers of APB binding, displacing 34 and 22%, respectively, of the binding at 1 mM (Table II).

Discussion

The purpose of this investigation was to compare the ligand specificity and the kinetic properties of this APB binding site to the pharmacological properties measured in the lateral perforant path of hippocampal slices. We also sought to verify that the binding site identified by our methods corresponds to the previously described Ca^{2+}/Cl^--dependent L-[^{3}H]-glutamate binding site. This binding site is enriched in synaptic plasma membrane fractions of rat brain (Fagg et al., 1982). The binding of DL-[^{3}H]APB was saturable, linear with protein concentration up to 250 μg of protein/mL, and stereoselective. From equilibrium studies, the racemic mixture of APB had a dissociation constant of 6.0 μM, and the maximum level of binding was 380 pmol/mg of protein. From kinetic studies the dissociation constant determined was 2.9 μM. These binding constants are similar to those reported in binding studies using DL-[^{3}H]APB as the ligand (Butcher et al., 1983; Monaghan et al., 1983) and binding studies in which L-[^{3}H]glutamate was displaced with DL-APB (Fagg et al., 1982). The maximum level of binding was higher than that previously reported in which L-[^{3}H]glutamate was used as the ligand. Foster et al. (1981b) have reported a B_{max} of 153 pmol/mg of protein under similar buffer conditions to those used in this study except that their assay was performed in the presence of 2.5 mM $CaCl_2$ rather than the 10 mM $CaCl_2$ used in this study. The same group reported that the 20 mM Cl^- ion concentration used in our study is near the concentration required to optimize this binding (Mena et al., 1982) and that the binding activity is more stable in HEPES-KOH buffer than in Tris-HCl, the buffer used by many investigators to examine glutamate binding sites (Foster et al., 1981a). We attribute the higher level of binding observed to these two factors. At this level of binding, a hypothetical binding site of 100 kilodaltons would be 3.8% of the synaptic plasma membrane protein. Although this is an unusually high level of binding when compared to other transmitter systems, it should be pointed out that quantitatively these other transmitters account for only a small fraction of the neurons in the central nervous system, while the excitatory amino acids are thought to account for a majority of the excitatory transmission in the central nervous system (Snyder & Bennett, 1976).

In agreement with Monaghan et al. (1983), this binding possesses many of the properties of the previously described Ca^{2+}/Cl^--dependent glutamate binding. The binding activity is abolished by freezing and is dependent on the presence of $CaCl_2$. In addition, this binding activity possesses similar ligand specificity to that previously observed. The binding is strongly displaced by quisqualate (IC_{50} = 0.26 μM), moderately sensitive to D-APV (IC_{50} = 15 μM), more sensitive to the L isomers of APB, glutamate, and aspartate than to the D isomers, and insensitive to kainate [see Foster & Fagg (1984)]. The sensitivity of this binding to DL-AMPB also agrees with that observed for the Ca^{2+}/Cl^--dependent glutamate binding site (Fagg et al., 1984).

In this investigation, we have extended the knowledge of the ligand specificity of this binding site using compounds that demonstrated a broad range of potencies for inhibiting synaptic transmission in the lateral perforant pathway. The potencies of these compounds in both assays are presented (see Tables I and II). Except for kynurenate, the rank order of those compounds identified as antagonists in the lateral perforant path parallels the rank order of these compounds as displacers of DL-[^{3}H]APB binding. The methyl-substituted derivatives of APB, DL-AMPB, β-methyl-APB, and γ-methyl-APB, were between 25- and 30-fold more potent as displacers of APB binding than as inhibitors of synaptic transmission at this APB-sensitive pathway (Table I). Binding was 20-fold more sensitive to D-APV than synaptic transmission is to DL-APV. α-Methyl-APB, a weak inhibitor of synaptic transmission, was approximately 10-fold more potent in the binding assay. Kynurenate, a postsynaptic antagonist (Ganong et al., 1983; Robinson et al., 1984a), was approximately 20-fold less potent as a displacer of binding than as an inhibitor of synaptic transmission. The agonists, L-glutamate tetrazole, L-glutamate, and D-glutamate, were between 140- and 7500-fold more potent in the binding assay. Baclofen was weak in both assays.

The binding appeared homogeneous by Scatchard analysis for the DL-[^{3}H]APB binding (Figure 1). Except for quisqualate, the displacement curves conformed to those expected for a homogeneous population of binding sites. The quisqualate displacement curve showed a 15% heterogeneity in the binding, and the kinetic analyses showed a 10% heterogeneity of a rapidly dissociating component (Figure 3). These data suggest that there may be a small heterogeneity in the binding sites observed. This minor rapidly dissociating component of binding is more comparable kinetically to the observations from electrophysiological experiments. In the hippocampal slice, the maximum level of L-APB-induced inhibition occurs within 2 min and remains constant thereafter, and the inhibited response completely recovers from the effects of APB within 4 min (Koerner & Cotman, 1981). These electrophysiological experiments put a lower limit on the kinetic rate constants because the drug must diffuse into and out of the slice. The

kinetic constants for the major component of binding determined by the binding assay are at least 25-fold lower than this lower limit.

We conclude that this binding does not represent binding to the recognition site of the native form or a "desensitized" form of the APB receptor in the lateral perforant path. If one only considers the parallel sensitivities of this binding and the responses in the lateral perforant path to the methyl-substituted APB analogues, this binding does appear to represent binding to an altered (desensitized) form of the receptor similar to that described for the nicotinic receptor. In the desensitized state, the nicotinic receptor displays altered kinetics which greatly increase the affinities for agonists and results in smaller differences in affinities for antagonists (Conti-Tronconi & Raftery, 1982). The parallel increases in potency of the methyl-substituted APB derivatives, which are small relative to the increases for the agonists, and decreases in kinetic rates suggest this may be a desensitized form of the receptor mediating the L-APB-induced inhibition of the lateral perforant path. However, two points tend to argue against this possibility. First, L-APB does not display a similar increase in potency in the binding assay. This would suggest the unlikely possibility that the "desensitization"-related changes in the protein conformation at the binding site resulted in an increase in the affinity for the displacers and slower kinetics for APB but had no effect on the affinity for APB. Another argument suggesting this binding protein is not the recognition site of this receptor stems from other electrophysiological experiments. The currently accepted "glutamate receptor" classification scheme includes three classes of receptors named for the most potent and specific agonist acting at each receptor subtype; these include the quisqualate receptor, the kainate receptor, and the *N*-methyl-D-aspartate receptor (Watkins & Evans, 1981). In the lateral perforant pathway (Ganong & Cotman, 1982) and the lateral olfactory cortex (Hori et al., 1982), APB reduces the excitation induced by kainate and *N*-methyl-D-aspartate and fails to affect the quisqualate-induced excitation. This is opposite to the ligand specificity of this binding which is most potently displaced by quisqualate and least potently displaced by kainate. It is possible that this binding protein is derived from a different receptor with some parallel pharmacological properties to that of the "L-APB" receptor.

Registry No. DL-APB, 20263-07-4; L-APB, 23052-81-5; D-APV, 79055-68-8; α-methyl-APB, 78405-44-4; β-methyl-APB, 95742-06-6; γ-methyl-APB, 79469-40-2; DL-AMPB, 53369-07-6; L-glutamate tetrazole, 65914-80-9; kynurenic acid, 492-27-3; L-glutamic acid, 56-86-0; D-glutamic acid, 6893-26-1; L-aspartic acid, 56-84-8; D-aspartic acid, 1783-96-6; kainic acid, 487-79-6; L-quisqualic acid, 52809-07-1.

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