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Interaction of [^{3}H]MK-801 with multiple states of the *N*-methyl-D-aspartate receptor complex of rat brain

(phencyclidine receptor/glycine receptor/serine/glutamate receptor/excitatory amino acids)

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ABSTRACT ***N*-Methyl-D-aspartate (*N*-Me-D-Asp) and phencyclidine receptors interactively mediate central nervous system processes including psychotomimetic effects of drugs as well as neurodegenerative, cognitive, and developmental events. To elucidate the mechanism of this interaction, effects of *N*-Me-D-Asp agonists and antagonists and of glycine-like agents upon binding of the radiolabeled phencyclidine receptor ligand [^{3}H]MK-801 were determined in rat brain. Scatchard analysis revealed two discrete components of [^{3}H]MK-801 binding after 4 hr of incubation. Incubation in the presence of L-glutamate led to an increase in apparent densities but not in affinities of both components of [^{3}H]MK-801 binding as well as conversion of sites from apparent low to high affinity. Incubation in the presence of combined D-serine and L-glutamate led to an increase in the apparent density of high-affinity [^{3}H]MK-801 binding compared with incubation in the presence of either L-glutamate or D-serine alone. These data support a model in which phencyclidine receptor ligands bind differentially to closed as well as open conformations of the *N*-Me-D-Asp receptor complex and in which glycine-like agents permit or factilitate agonist-induced conversion of *N*-Me-D-Asp receptors from closed to open conformations.**

Phencyclidine [*N*-(1-phenylcyclohexyl)piperidine; PCP] and related drugs bind to a specific class of PCP receptors and exert unique neurobehavioral (1) and neuroprotective (2) effects. Several lines of evidence indicate that the PCP receptor represents a site within the ion channel gated by the *N*-methyl-D-aspartate (*N*-Me-D-Asp) excitatory amino acid receptor. PCP and *N*-Me-D-Asp receptors are co-localized in brain (2–5). PCP-like drugs noncompetitively antagonize *N*-Me-D-Asp receptor-mediated electrophysiological effects in proportion to their potencies in binding to PCP receptors (6, 7) in a use- (2, 8–10) and voltage- (9, 10) dependent manner. Radioligand binding to PCP receptors is stimulated by *N*-Me-D-Asp receptor agonists (11–17) and can be antagonized by the competitive *N*-Me-D-Asp antagonist D-(−)-2-amino-5-phosphonovaleric acid [D(−)AP5] (13–19). Glycine- or D-serine-like amino acids stimulate radioligand binding to PCP receptors by a mechanism distinct from that of L-glutamate (16–18). It has been proposed that PCP-like compounds bind selectively to the open (8–10) activated (13, 14) *N*-Me-D-Asp receptor channel complex. However, some binding of PCP-like agents has been shown to occur even under conditions in which little if any receptor activation would occur [e.g., absence of added *N*-Me-D-Asp receptor agonists or presence of D(−)AP5] (20, 21). (+)-5-Methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohept-5,10-imine maleate (MK-801) has been shown to interact competitively with PCP receptor ligands (22, 23). For the present study [^{3}H]MK-801 binding was characterized under various degrees of *N*-Me-D-Asp receptor activation. Our results suggest that PCP-like drugs bind to closed as well as open conformations of the *N*-Me-D-Asp receptor channel complex.

MATERIALS AND METHODS

Crude synaptic membranes were prepared by the method of Zukin *et al.* (24). Male Long–Evans rats (200–250 g) were decapitated. Cerebral cortex plus hippocampus was rapidly removed and homogenized in 15 vol of ice-cold 0.32 M sucrose by using a Teflon–glass homogenizer at 800 rpm. The homogenate was centrifuged at 1000 × *g* for 10 min in a Sorvall RC-5B refrigerated centrifuge. The supernatant was decanted and centrifuged at 20,000 × *g* for 20 min. The resulting pellet was suspended in 30 vol of deionized water by using a Brinkman Polytron, setting 6; the resulting suspension was centrifuged at 8000 × *g* for 20 min. The supernatant and buffy coat were decanted and centrifuged at 48,000 × *g* for 20 min. The pellet was then resuspended in 5 mM EDTA buffered to pH 7.4 by the addition of Tris base (≈15 mM) and incubated for 1 hr at 37°C. After incubation, the membrane suspension was again centrifuged at 48,000 × *g* for 20 min. Pellets were then frozen at −4°C for 1–14 days.

On the day of experiment, frozen pellets were thawed and suspended in 30 vol of deionized water for 1 hr at 37°C. The membrane suspension was re-centrifuged for 20 min at 48,000 × *g*. Pellets were then washed three times by resuspension in 30 vol of deionized water and centrifugation at 48,000 × *g*. After the final centrifugation, pellets were suspended in 30–50 vol of 5 mM Tris acetate adjusted to pH 7.4. Aliquots (1 ml) of membrane homogenate were incubated for 4 hr with various concentrations of [^{3}H]MK-801 between 0.5 nM and 3 μM. Nonspecific binding was determined in the presence of 100 μM *N*-(1-[2-thienyl]cyclohexyl)piperidine (TCP). *N*-Me-D-Asp, L-glutamate, glycine, and D-serine were obtained from Sigma. D(−)AP5 was obtained from Cambridge Research Biochemicals (Long Beach, NY). [^{3}H]MK-801 was obtained from Dupont/NEN. Incubations were terminated by filtration under reduced pressure in a 24-well cell harvester (Brandel, Gaithersburg, MD) and Whatman GF/B filters that had been soaked for 30 min in 0.3% polyethyleneimine. Filter disks were placed in vials containing 4 ml of Hydrofluor (National Diagnostics, Manville, NJ) and radioactivity was measured by using an LKB 1218 Rackbeta scintillation counter at a counting efficiency of 50%.

Data analysis was performed with the Scafit nonlinear curve-fitting program (generously provided by Peter J. Munson, National Institute of Child Health and Human Development) on an IBM PC/XT computer. For the results of each saturation experiment, one-, two-, and three-site fits were attempted. In each case, analyses were carried out using both

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Abbreviations: *N*-Me-D-Asp, *N*-methyl-D-aspartate; PCP, phencyclidine; D(−)AP5, D-(−)-2-amino-5-phosphonovaleric acid.
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fixed and computer-determined values for nonspecific binding. In the absence of a significant difference in goodness-of-fit ($p<0.05$), fits of lower complexity were used for subsequent analyses.

RESULTS

Parameters of [^{3}H]MK-801 binding were determined by computer-assisted Scatchard analysis (Table 1 and Fig. 1). In the absence of added L-glutamate or D-serine binding of [^{3}H]MK-801 occurred primarily (>95%) to an apparent low-affinity component (apparent K_d = 34 ± 4 nM). An apparent high-affinity component (apparent K_d = 0.8 ± 0.4 nM) accounted for the remainder of binding (<5%). The binding parameters of [^{3}H]MK-801 determined under baseline conditions were compared to those determined in the presence of L-glutamate, D(−)AP5, or D-serine. None of these conditions resulted in a significant variation in apparent K_d values of apparent high- (K_H) or low- (K_L) affinity components of binding as determined by single factor analysis of variance testing (K_H: $F_{6,26}$ = 2.08, NS. K_L: $F_{5,22}$ = 3.12, NS). The mean K_d values for the apparent high- and low-affinity components of binding under all conditions were 2.2 ± 0.3 nM and 40 ± 7 nM, respectively. By contrast, the calculated densities of the apparent high- (R_H) and low- (R_L) affinity components of binding varied significantly between groups (R_H: $F_{6,24}$ = 20.92, $P < 0.0001$. R_L: $F_{5,19}$ = 4.47, $P < 0.05$).

When [^{3}H]MK-801 binding was carried out in the presence of added L-glutamate alone, binding data consistently were fit better by a two-component (high- and low-affinity components) than by a one-component model. Incubation in the presence of 100 nM, 500 nM, or 10 μM L-glutamate led to a significant increase in the calculated density of the apparent high-affinity component (R_H) above baseline. The density of the apparent low-affinity component of binding (R_L) was significantly higher in the presence of 10 μM L-glutamate than at baseline. The total density (R_{TOT}) was significantly increased above baseline by all tested concentrations of added L-glutamate. The density of the apparent high-affinity component of binding accounted for a progressively larger percentage of the total density as the concentration of L-glutamate was increased.

Incubation in the presence of 10 μM D-serine alone led to a small but significant increase in the density of the high-affinity component of binding above baseline. Nonsignificant increases in apparent low-affinity ($P < 0.2$) and total ($P < 0.1$) binding were also observed. After incubation in the presence of combined 10 μM D-serine and 10 μM L-glutamate, a highly significant increase in the density of the apparent high-affinity component above baseline was observed and no apparent low-affinity component was detected. A significant increase in the total density above baseline was also observed. The density of the apparent high-affinity component of binding was significantly greater in the presence of combined D-serine and L-glutamate than in the presence of either D-serine or L-glutamate alone.

Incubation in the presence of 10 μM D(−)AP5 led to a significant decrease in the density of the apparent low-affinity component of binding ($P < 0.05$) and in the total density ($P < 0.05$) as compared with baseline. The densities of the apparent high- (0.27 ± 0.15 pmol/mg of protein) and low- (0.67 ± 0.66 pmol/mg of protein) affinity components of binding were not significantly different from 0. The apparent K_d values of the apparent high- (2.8 ± 0.66 nM) and low- (20.8 ± 7.4 nM) affinity components of binding were not significantly different in the presence of D(−)AP5 than at baseline.

In summary, under six of seven conditions, [^{3}H]MK-801 binding fit significantly better to a two-component than to a one-component model. The presence of distinct components of [^{3}H]MK-801 binding suggests the existence either of two separate sites for [^{3}H]MK-801 binding or of two separate states of a single binding site. In the presence of 10 μM D-serine, L-glutamate significantly increased [^{3}H]MK-801 binding to the apparent high- vs. the apparent low-affinity component without affecting the total density of binding sites. This apparent interconversion suggests that the two components of [^{3}H]MK-801 binding represent separate states of a single binding site (PCP receptor).

DISCUSSION

The major finding of the present study is that, after 4 hr of incubation, [^{3}H]MK-801 binding manifests two apparent sites that have different affinity constants and whose densities are affected by *N*-Me-D-Asp receptor activation. This suggests that PCP-like agents cannot be conceptualized as interacting only with a single activated state of the *N*-Me-D-Asp receptor complex since such binding would be expected to manifest only a single component (25). Previous studies with [^{3}H]MK-801 have not reported multiple components of binding. Those studies, however, were conducted under conditions (baseline or in the presence of combined glutamate and glycine) in which a single state predominated in the present study (14, 21) or did not specify the number of data points or concentration range of Scatchard analysis (16).

Association and dissociation kinetics of PCP receptor ligands are slower in the absence than in the presence of *N*-Me-D-Asp receptor agonists (20, 21, 26). The possibility must be considered whether such kinetic factors alone could account for the findings reported here. This seems unlikely. We have found specific binding of 1 nM [^{3}H]MK-801 to attain up to 86% of its ultimate steady-state value after 4 hr of incubation under baseline conditions (D.C.J., M. J. Frusciante, and S.R.Z., unpublished data). The $t_{1/2}$ of dissociation for [^{3}H]MK-801, which equals its maximal $t_{1/2}$ of association, has been reported to be 40 min (26) or between 70 and 180 min

Table 1. [^{3}H]MK-801 binding parameters

	Dissociation constant, nM		Apparent density, pmol/mg of protein			
	K_H	K_L	R_H	R_L	R_{TOT}	$\%R_H$
Baseline	0.8 ± 0.4	34 ± 4	0.06 ± 0.04	3.0 ± 0.6	3.1 ± 0.6	1.6 ± 1.0
L-Glutamate (100 nM)	2.8 ± 1.5	80 ± 21	1.2 ± 0.5‡	6.0 ± 1.8	7.1 ± 1.4*	15.0 ± 4.9‡
L-Glutamate (500 nM)	3.8 ± 0.8	77 ± 43	2.2 ± 0.9‡	5.3 ± 1.8	7.5 ± 2.1*	28.2 ± 11.8†
L-Glutamate (10 μM)	2.0 ± 0.3	30 ± 15	2.1 ± 0.7‡	6.3 ± 1.3*	8.4 ± 1.5‡	25.0 ± 7.0‡
D-Serine (10 μM)	1.2 ± 0.1	12 ± 4	0.32 ± 0.09†	5.3 ± 1.4	5.6 ± 1.1	6.0 ± 2.6
D-Serine (10 μM) + L-glutamate (10 μM)	2.1 ± 0.6	—	7.8 ± 1.6‡	—	7.8 ± 1.6†	100

Apparent dissociation constants and densities of components of [^{3}H]MK-801 binding were derived by computer-assisted Scatchard analysis. Values represent means ± SEM of between three and eight experiments each performed in triplicate.
*$P < 0.05$ vs. baseline.
†$P < 0.01$ vs. baseline.
‡$P < 0.001$ vs. baseline.

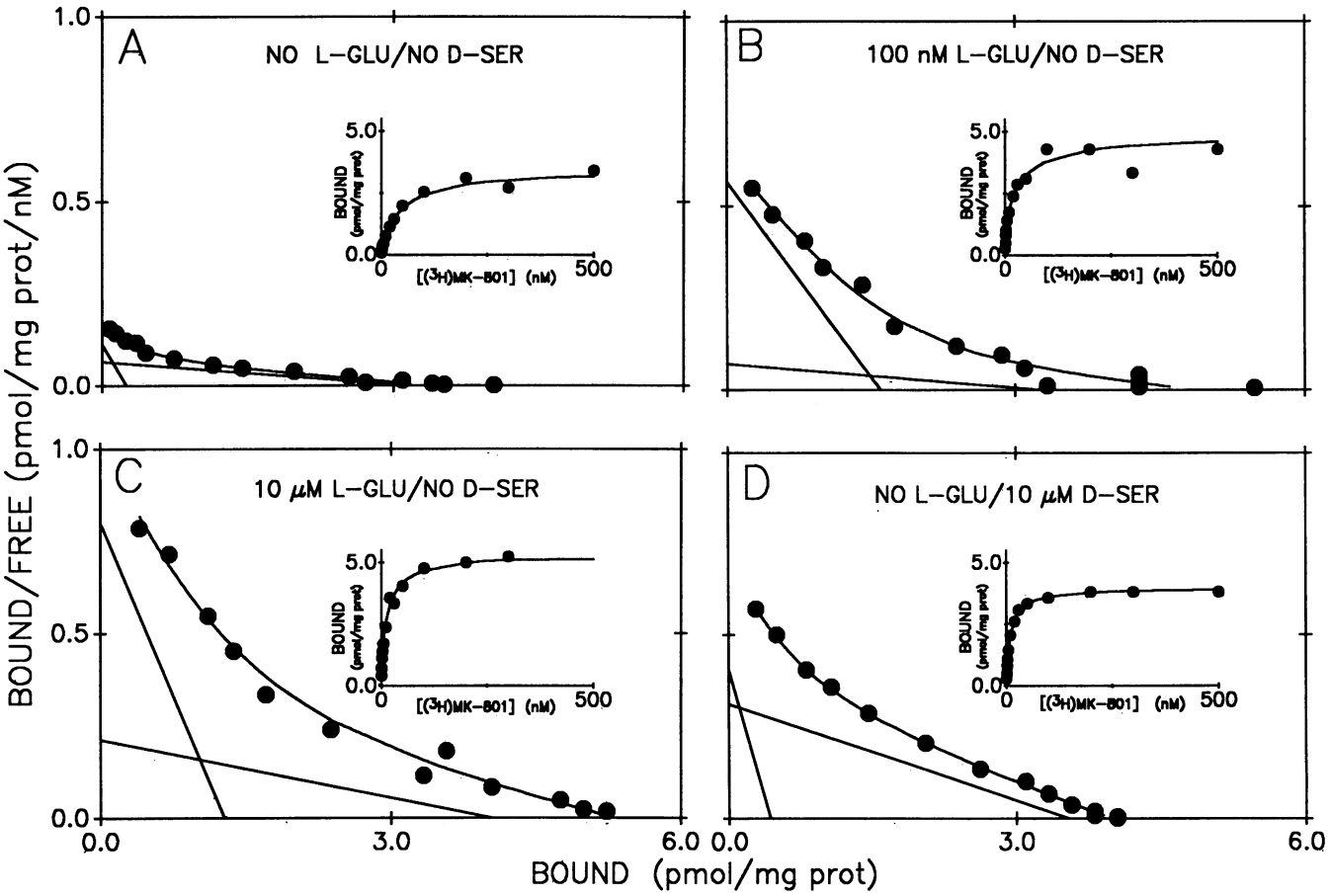

FIG. 1. Representative Scatchard plots of specific [^{3}H]MK-801 binding in the absence and presence of L-glutamate and D-serine. Various concentrations of [^{3}H]MK-801 between 0.5 nM and 3 μM were tested. Each point was determined in triplicate. Straight lines represent computer-determined apparent high- and low-affinity components of binding.

(20) in the absence of added agonist and 18 min in the presence of L-glutamate (20). From that data it can also be calculated that after our incubation of 4 hr, [^{3}H]MK-801 binding should have attained >80% of its ultimate equilibrium value under baseline conditions and >99% in the presence of L-glutamate. Failure to reach true steady state, moreover, could lead to overestimation of K_d values derived from Scatchard analysis (27, 28) but would not lead to a single state appearing to be multiple discrete states.

[^{3}H]MK-801 can become "trapped" inside open *N*-Me-D-Asp receptor channels when they close upon removal of agonist (9, 10). Thus the question arises whether such trapping might account for our observation of dual components of [^{3}H]MK-801 binding. Based upon the forward rate constant for MK-801 binding to *N*-Me-D-Asp receptor channels of 3×10^7 $M^{-1} \cdot sec^{-1}$ (10), association of 1 nM MK-801 might be slow compared to mean channel open time (29–31). Under such circumstances the influences of a "guard function" (reflecting the fraction of unbound sites to which ligand has access) and of a "trap function" (reflecting the fraction of bound sites from which ligand can freely escape) may result in overestimation of K_d but will not affect the determination of B_{max} values or lead to the appearance of a second component of binding (25); such considerations, therefore, would not result in a single state appearing to be multiple discrete states. Thus, neither kinetic-barrier nor transient-access models of PCP/*N*-Me-D-Asp receptor interaction appear to account adequately for the present data.

The apparent interconversion of two discrete components of [^{3}H]MK-801 binding observed in the present study suggests that [^{3}H]MK-801 can bind to both open and closed conformations of the *N*-Me-D-Asp receptor complex. The variation in the relative densities of apparent high- and low-affinity components under different experimental conditions suggests that [^{3}H]MK-801 itself does not prevent interconversion between open and closed conformations. This observation is consistent with recent electrophysiological evidence demonstrating that MK-801 does not prevent *N*-Me-D-Asp channel closure (9, 10). Under conditions that would be expected to lead to maximal *N*-Me-D-Asp receptor activation (i.e., added L-glutamate plus D-serine) all binding of [^{3}H]MK-801 occurred with apparent high affinity, suggesting that the apparent high-affinity component of binding represents binding to the open conformation of the *N*-Me-D-Asp receptor complex. The apparent K_d of high-affinity [^{3}H]MK-801 binding determined in the present study [2.2 ± 0.3 nM (mean ± SEM) over all conditions] corresponds well with the reported IC_{50} of 1.47 nM for MK-801-induced inhibition of *N*-Me-D-Asp receptor-mediated neurotransmitter release from hippocampal slices (22) and with the reported potency of MK-801 for inducing open channel blockade (8, 10). By contrast, in the absence of added L-glutamate or D-serine virtually all sites (>98%) bound [^{3}H]MK-801 with apparent low affinity, suggesting that the apparent low-affinity component represents binding of [^{3}H]MK-801 to closed conformations of the *N*-Me-D-Asp receptor complex. The simultaneous appearance of high- and low-affinity components of binding in the presence of intermediate degrees of *N*-Me-D-Asp receptor stimulation (L-glutamate or D-serine alone) suggests that separate processes, such as association through distinct paths (25), must underlie the interaction of [^{3}H]MK-801 with open vs. closed *N*-Me-D-Asp receptor complexes.

In addition to increasing the density of the apparent high-affinity component of [^{3}H]MK-801 binding, incubation with L-glutamate led to a significant increase in total receptor density above baseline. This finding suggests that under baseline conditions, a population of non-agonist-associated receptor complexes exists to which binding of [^{3}H]MK-801 was not detected under our experimental conditions, perhaps because the binding affinity of this component is beyond the limits of detection of the rapid filtration assay that we employed. The increase in density of the apparent low-affinity [^{3}H]MK-801 binding component induced by addition of L-glutamate indicates that it corresponds to a closed agonist conformation of the *N*-Me-D-Asp receptor complex. The existence of closed agonist-associated receptors is consistent with the report (32) that *N*-Me-D-Asp receptor agonists fail to produce channel opening in the total absence of glycine. The detection of apparent high-affinity binding in the absence of added D-serine in the present study may reflect the existence of residual endogenous glycine-like agents in our membrane preparation.

A final effect of L-glutamate was to increase the percentage of *N*-Me-D-Asp receptor complexes to which [^{3}H]MK-801 bound with apparent high rather than low affinity (% R_H). The L-glutamate-induced increase in the % R_H value suggests that after L-glutamate-induced conversion of complexes from the nondetected form to the form manifesting low-affinity [^{3}H]MK-801 binding, binding of at least one additional molecule of L-glutamate is required before conversion of complexes from apparent low to high affinity can occur. However, incubation with L-glutamate alone did not lead to complete conversion of receptor complexes from apparent low to high affinity, suggesting that binding of L-glutamate alone may not be sufficient for *N*-Me-D-Asp receptor activation. By contrast, incubation with combined L-glutamate and D-serine did lead to complete conversion. Both L-glutamate and D-serine may thus be required for activation of the *N*-Me-D-Asp receptor complex.

A model (Fig. 2) that could account for our findings is one which assumes the *N*-Me-D-Asp receptor complex can bind two molecules of L-glutamate independently. Only the form of the complex that has bound two molecules of L-glutamate (RL_2) would be capable of channel opening. Conversion of this form to the open conformation (AL_2) would depend upon a process that is independent of L-glutamate but might depend upon binding of glycine-like agents. It is also assumed that interconversion between different binding states is slow compared to MK-801 equilibration.

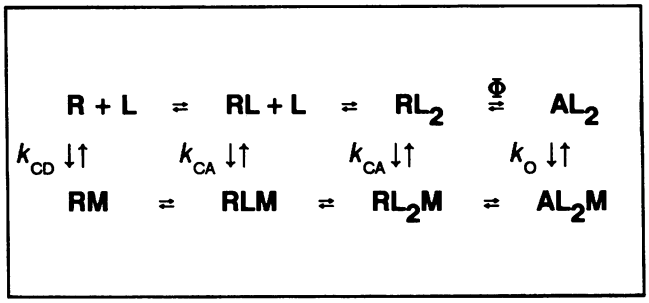

FIG. 2. Four-state model of receptor conformation (33–35). R represents a resting (closed) agonist-dissociated receptor conformation. RL and RL_2 represent resting (closed) agonist-associated conformations. AL_2 represents the activated (open) receptor conformation. L represents ligand (i.e., L-glutamate or *N*-Me-D-Asp). M represents [^{3}H]MK-801. Equilibria between [^{3}H]MK-801 dissociated forms (R, RL, RL_2, and AL_2) and [^{3}H]MK-801 associated forms (RM, RLM, RL_2M, and AL_2M) depend upon the dissociation constants (K_{CD}, K_{CA}, and K_O) for [^{3}H]MK-801 binding to the closed agonist-dissociated (R), closed agonist-associated (RL and RL_2), and open (AL_2) conformations. It is assumed that binding of [^{3}H]MK-801 does not prevent the dynamic equilibrium between agonist-associated and -dissociated or between resting and activated conformations of the receptor complex. [^{3}H]MK-801 association with the closed agonist-dissociated state (R) was not detected under our experimental conditions. Apparent low-affinity [^{3}H]MK-801 binding corresponds to the closed agonist-associated states (RL and RL_2), whereas apparent high-affinity [^{3}H]MK-801 binding corresponds to the open state (AL_2). Φ, the conformational equilibrium constant, is defined as $[RL_2]/[AL_2]$. CA, closed ligand-associated; CD, closed ligand-dissociated; O, open.

Predicted relative densities for each of the closed conformations (R, RL, and RL_2) of the proposed model were calculated (Table 2) based upon the reported endogenous concentration of L-glutamate in crude synaptic membranes and the reported affinity of L-glutamate for *N*-Me-D-Asp receptors. The effective concentration of free L-glutamate in well-washed crude synaptic membrane fractions similar to those employed in this study can be calculated to range between 5 and 25 nM (14). Our observation that D(−)AP5 inhibited baseline binding of [^{3}H]MK-801 further supports the concept that low concentrations of residual excitatory amino acids are present in our membrane preparation. The affinity of L-glutamate for the *N*-Me-D-Asp receptor has been reported to be 100–300 nM (12–14, 36). Assuming an effective free concentration of L-glutamate of 15 nM and an affinity of 100 nM for L-glutamate binding to *N*-Me-D-Asp receptors, the predicted relative density of receptor complexes in the non-agonist-associated closed conformation (R) under each experimental condition correlated well [correlation coefficient (r) = 0.92, degrees of freedom (df) = 4, $P < 0.02$] with the relative density of sites not detectable by [^{3}H]MK-801 binding (Table 3). Furthermore, the predicted relative density of receptors in the agonist-associated closed conformations (RL + RL_2) correlated well ($r = 0.83$, df = 4, $P < 0.05$) with the observed relative density of the apparent low-affinity component of [^{3}H]MK-801 binding (Table 3). The overall correlation between predicted and observed values was highly significant ($r = 0.82$, df = 10, $P < 0.001$). Correlation

Table 2. Predicted relative densities of *N*-Me-D-Asp receptor conformations

Condition	[Glu]/K_d(Glu)	Relative density R	RL + RL_2	Φ
Baseline	0.2	0.75	0.24	2.41
L-Glutamate (100 nM)	1.2	0.21	0.64	1.44
L-Glutamate (500 nM)	5.2	0.02	0.71	1.86
L-Glutamate (10 μM)	100.2	0.00	0.75	2.94
D-Serine (10 μM)	0.2	0.73	0.23	0.41
D-Serine (10 μM) + L-glutamate (10 μM)	100.2	0.00	0.04	0.04

Densities of closed receptor conformations of the proposed model (Fig. 2) were predicted from total density in the closed conformations and the formula $K_d(Glu) = 2[R][Glu]/[RL] = [RL] \times [Glu]/2[RL_2]$, where K_d(Glu) represents the dissociation constant for L-glutamate binding to *N*-Me-D-Asp receptors, [Glu] represents the concentration (endogenous + added) of L-glutamate, [R] represents the density of receptor complexes in the closed conformation not associated with agonist (L-glutamate), [RL] represents the density of receptor complexes in the closed conformation associated with a single molecule of L-glutamate, and $[RL_2]$ represents the density of receptor complexes in the closed conformation associated with two molecules of L-glutamate (33, 35). The total density of receptor complexes in the closed conformation was calculated by subtracting R_H (Table 1) from the maximal receptor density (taken as 8.1 pmol/mg of protein). Relative densities (R, RL, and RL_2) were determined by dividing [R], [RL], and $[RL_2]$ by 8.1 pmol/mg of protein. An endogenous glutamate concentration of 15 nM and a K_d value of 100 nM for glutamate binding to *N*-Me-D-Asp receptors were assumed. Values for Φ were determined by dividing the predicted relative density of receptor complexes in the RL, conformation (values not shown separately) by the observed relative density of high-affinity [^{3}H]MK-801 binding sites (Table 3).

Table 3. Observed relative densities of states of [^{3}H]MK-801 binding

Condition	Relative density		
	N.D.	Low	High
Baseline	0.62	0.37	0.01
L-Glutamate (100 nM)	0.11	0.74	0.15
L-Glutamate (500 nM)	0.07	0.65	0.28
L-Glutamate (10 μM)	0.00	0.78	0.26
D-Serine (10 μM)	0.31	0.65	0.04
D-Serine (10 μM) + L-glutamate (10 μM)	0.04	0.00	0.96

Relative densities of apparent low- and high-affinity components of [^{3}H]MK-801 binding were determined by dividing densities of apparent low- and high-affinity binding (Table 1) by the average of the apparent densities determined in the presence of saturating L-glutamate alone and in the presence of saturating L-glutamate and D-serine (8.1 pmol/mg of protein). The relative density of nondetected (N.D.) sites was determined by subtracting the sum of the relative densities of low- plus high-affinity binding sites from 1.00.

between predicted and observed values remained significant even if estimates for the concentration of endogenous L-glutamate and for the affinity of L-glutamate binding to *N*-Me-D-Asp receptors were permitted to vary throughout the ranges of their reported values. Overestimation of K_d values due to the kinetic or transient-access considerations detailed above would not impair the significance of the correlation because, as detailed above, such considerations would not influence calculated densities of the states.

In the proposed model, the ratio of RL_2 to AL_2 conformations is represented by Φ, a conformational equilibrium constant (Fig. 2). Values of Φ under each experimental condition (Table 2) can be estimated by dividing the predicted relative density of receptor complexes in the two agonist-associated closed conformation (RL_2) by the observed relative density of [^{3}H]MK-801 binding sites in the high-affinity state (AL_2). The calculated mean value of Φ is significantly lower in the presence (0.23 ± 0.19) than in the absence (2.16 ± 0.28) of added D-serine ($P < 0.01$). This observation is consistent with a model in which glycine-like agents regulate channel opening by interaction with a site that is independent of the L-glutamate binding site.

Our hypothesis that differential binding of [^{3}H]MK-801 to RL, RL_2, and AL_2 conformations results in distinct components on Scatchard analysis was based upon the assumption that interconversion between these states is slow compared to [^{3}H]MK-801 equilibration with each state. However, the $t_{1/2}$ value of dissociation of L-glutamate is about 2 min (36), and the $t_{1/2}$ value for dissociation of glycine is about 0.5 min (37), while dissociation of MK-801 is much slower with or without agonist (20, 26). In electrophysiological experiments a τ value of 1.8 min (which represents an upper limit for the $t_{1/2}$ of MK-801 dissociation) was reported for *N*-Me-D-Asp channels to recover in the presence of agonist from MK-801-induced blockade at +30 mV (10). In these experiments entry of MK-801 into closed channels is much slower, and recovery in the absence of agonist is also very slow. The radically different environments of receptors on intact cells and in membrane homogenates make comparisons between electrophysiological and binding data problematic.

It is interesting to note that multistate models analogous to the one proposed here, requiring two molecules of agonist for channel activation, may account for the functioning of both nicotinic cholinergic (33, 34) and $GABA_A$ (35) receptors (where GABA is γ-aminobutyric acid). Both nicotinic and $GABA_A$ receptors have been shown to undergo desensitization (33–35), a process that has also been reported in electrophysiological paradigms for *N*-Me-D-Asp receptors (9, 10). Although our proposed model does not account for all aspects of *N*-Me-D-Asp receptor functioning, a more general model of *N*-Me-D-Asp receptor function must account for the multiple interconverting states that we observed.

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