0026-895X/89/060887-10$02.00/0

MOLECULAR PHARMACOLOGY, 36:887-896

The Psychotomimetic Drug Phencyclidine Labels Two High Affinity Binding Sites in Guinea Pig Brain: Evidence for *N*-Methyl-D-aspartate-Coupled and Dopamine Reuptake Carrier-Associated Phencyclidine Binding Sites

RICHARD B. ROTHMAN, AUDREY A. REID, JAMES A. MONN, ARTHUR E. JACOBSON, and KENNER C. RICE

Unit on Receptor Studies, LCS, National Institute of Mental Health (R.B.R.) and Laboratory of Medicinal Chemistry, National Institute of Diabetes, Digestive and Kidney Diseases (A.A.R., J.A.M., A.E.J., K.C.R.) Bethesda, Maryland 20892

Received March 24, 1989; Accepted September 14, 1989

SUMMARY

Numerous studies have now demonstrated that a binding site for the psychotomimetic drug phencyclidine (PCP) exists within the receptor channel complex for the excitatory amino acid neurotransmitter glutamate, specifically the glutamate receptor selectively activated by *N*-methyl-D-aspartate (NMDA). Several lines of evidence support the hypothesis that all PCP receptors in rat brain are associated with the NMDA receptor complex. In the present study, we reexamine this hypothesis. We report that the PCP analog [^{3}H]1-[1-(2-thienyl)cyclohexyl]piperidine ([^{3}H]TCP) labels two high affinity binding sites in membranes prepared from guinea pig brain site 1 (K_d = 14.1 nM, B_{max} = 631 fmol/mg of protein) and site 2 (K_d = 46.5 nM, B_{max} = 829 fmol/mg of protein). (+)-5-Methyl-10 11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine maleate bound to site 1 with high affinity (K_i = 3.2 nM) and to site 2 with low affinity (K_i = 5208 nM). The order of potency of drugs for inhibiting [^{3}H]TCP binding to site 1 correlated with their ED_{50} values for inhibition of NMDA-mediated responses reported in the literature, whereas the order of potency of drugs for inhibiting [^{3}H]TCP binding to site 2 correlated with their ED_{50} values for inhibition of [^{3}H]dopamine reuptake reported in the literature. Kinetic experiments demonstrated that glutamate, 2-amino-7-phosphonoheptanoic acid, and Mg^{2+} modulated [^{3}H]TCP binding to site 1 but not site 2. Preincubation of guinea pig striatal membranes with varying concentrations of the high affinity dopamine reuptake inhibitors *N*-[1-(2-benzo(*b*)thiophenyl)cyclohexyl]piperidine and 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-[3-phenylpropyl]piperazine caused a wash-resistant inhibition of [^{3}H]TCP binding to site 2 but not site 1. Taken collectively, these data demonstrate the existence of a high affinity PCP binding site associated with the dopamine reuptake carrier and raise the possibility that the therapeutic and psychotomimetic effects of PCP in humans are separable and mediated via different binding sites.

PCP is an arylcyclohexylamine originally developed in 1958 as a dissociative anesthetic (1). However, when it became apparent that PCP produced a variety of dysphoric and psychotic effects (2, 3), its use as a therapeutic agent in humans ceased. Subsequently, starting in 1965 and continuing to the present time, the use of PCP as a recreational drug of abuse has become a public health problem of national proportions (4).

Specific PCP binding sites for [^{3}H]PCP were identified in 1979 in rat brain (5, 6). The potency with which many analogs of PCP displace [^{3}H]PCP or its analog [^{3}H]TCP from PCP binding site(s) is highly correlated with their potencies in behavioral, drug discrimination, and *in vitro* functional assay systems (7–9), strengthening the hypothesis that the PCP binding site assayed *in vitro* might function as a receptor *in vivo*. The PCP-like drugs that produce psychotic and dysphoric effects in humans, as well as at least one of the enantiomers of the psychotomimetic opioids cyclazocine, pentazocine, and SKF10,047 (6, 10), have been found to interact with both PCP binding sites and with dopaminergic systems (11), the latter being a neuronal system implicated in the pathogenesis of schizophrenia (12). These data support the hypothesis that the PCP receptor system might be involved in the pathogenesis of certain mental illnesses and, therefore, that PCP-induced psychosis might be a relevant model for the study of these diseases (13, 14).

The seminal finding that PCP and the related dissociative anesthetic ketamine acted as noncompetitive antagonists of the excitatory amino acid neurotransmitter glutamate (15) led to a series of investigations that demonstrated that the PCP recep-

ABBREVIATIONS: PCP, phencyclidine; BTCP, *N*-[1-(2-benzo(*b*)thiophenyl)cyclohexyl]piperidine; GBR12909, 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-[3-phenylpropyl]piperazine; TCP, 1-[1-(2-thienyl)cyclohexyl]piperidine; AP7, 2-amino-7-phosphonoheptanoic acid; NMDA, *N*-methyl-D-aspartate; (+)-MK801, (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine maleate; DA, dopamine; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,-*N*′,*N*′-tetraacetic acid.

tor and the glutamate receptor subtype selectively activated by NMDA (NMDA receptor) coexist in a receptor/ionophore complex. Of the key observations supporting this hypothesis, the two most relevant to the present study are that agonists and competitive antagonists of the NMDA receptor modulate the PCP binding site *in vitro* (16, 17) and that high affinity PCP receptor ligands act as potent noncompetitive antagonists of the NMDA receptor (18).

PCP interacts with several central nervous system receptors/macromolecules. These include the potassium channel (19), the DA reuptake carrier (20), the haloperidol-sensitive σ receptor (21), the nicotinic receptor (22), and the NMDA receptor (15, 18). Several lines of evidence support the hypothesis that the NMDA receptor may be the most pharmacologically relevant of these putative PCP receptors: 1) PCP inhibits NMDA-mediated effects at nanomolar concentrations *in vitro* (23, 24); 2) most ligand binding studies detect only a single class of high affinity PCP binding sites, which have an anatomical distribution almost identical to that of the NMDA receptor (25); 3) (+)-MK801, which is almost inactive as an inhibitor of DA reuptake (26), produces many of the same behavioral effects as PCP (27, 28) and has a high affinity for PCP binding sites (29); 4) NMDA receptor agonists and antagonists modulate [^{3}H]TCP binding (16, 17); and 5) animals trained to discriminate PCP from saline generalize to (+)-MK801 and other noncompetitive NMDA antagonists (30, 31).

Nevertheless, other data suggest that all pharmacologically relevant PCP receptors might not be coupled to the NMDA receptor. For example, animals trained to discriminate PCP from saline generalize partially, or not at all, to competitive NMDA antagonists (32, 33). Furthermore, although competitive NMDA antagonists and (+)-MK801 produce many of the same behavioral effects in rats as does PCP, the precise relationship between behavioral measures such as catalepsy and ataxia to psychotomimesis remains to be defined. Moreover, relatively low doses of PCP increase extracellular levels of DA, as measured by *in vivo* microdialysis (34), whereas (+)-MK801 has no effect.[1] More importantly, in one study, at doses sufficient to control seizures, (+)-MK801 was not reported to be psychotomimetic (35), supporting the hypothesis that the ability of PCP to elevate extracellular levels of DA might be critical to its psychotomimetic properties.

The observation that benzomorphan opiates and (+)-MK801 bind potently to the PCP binding site yet are almost inactive as inhibitors of DA reuptake (24, 26) is evidence that the high affinity PCP binding site commonly labeled in rat brain is associated with the NMDA receptor and is not associated with the DA reuptake carrier. However, the good correlation between the affinity of arylcycloalkylamines for the high affinity PCP binding site and their ED_{50} values for inhibition of DA reuptake (20, 23), led us to examine the hypothesis that ligand binding studies should detect two high affinity PCP binding sites, the PCP receptor of the NMDA/ionophore receptor complex (site 1) and a high affinity PCP receptor associated with the DA reuptake carrier (site 2).

To test this hypothesis, we formulated the following predictions: 1) quantitative analysis of the interaction of (+)-MK801 and PCP with [^{3}H]TCP binding should resolve two high affinity binding sites, with (+)-MK801 being highly selective for site 1 and PCP being nonselective between the two sites; 2) NMDA receptor agonists and antagonists should modulate site 1, but not site 2; 3) preincubation of membranes with high affinity DA reuptake inhibitors should produce a wash-resistant inhibition of [^{3}H]TCP binding to site 2 but not site 1; and 4) the affinity of drugs for site 2 should correlate with their ability to inhibit the reuptake of DA.

[1] A. Pert, personal communication.

Materials and Methods

Preparation of membranes. Because our initial binding studies with guinea pig brain membranes readily resolved two high affinity binding sites (36), the present study was conducted using membranes prepared from guinea pig brain. Large batches of frozen membranes were prepared with minor modifications of published procedures (16). Twenty to 30 frozen guinea pig brains with cerebellum were homogenized with a Polytron in ice-cold 5 mM Tris·HCl, pH 8.2 (buffer, 10 ml/brain). The homogenate was centrifuged at 37,000 × *g* for 10 min, and the pellet was washed by resuspension in the same volume of buffer followed by recentrifugation. The pellets were resuspended with buffer (10 ml/brain) and incubated for 15 min at 0°. The buffer concentration was adjusted to 50 mM by the addition of 1 M Tris·HCl, pH 8.2, and the homogenate was centrifuged for 10 min at 15,000 × *g*. The pellets were then washed three times by centrifugation using 50 mM Tris·HCl, pH 8.2. The final pellets were resuspended in buffer (0.75 ml/brain) and pooled together, and 1-ml aliquots were distributed to microfuge tubes, which were stored at −70° for assay.

Binding assays. [^{3}H]TCP binding assays proceeded according to published protocols (37). Briefly, incubations proceeded, in a final volume of 1 ml, for 3 to 4 hr at 0° in 5 mM Tris·HCl, pH 8.2, containing about 1 mg of membrane protein, 10 μM EDTA, 10 μM EGTA, and 2.5 μg of the protease inhibitors chymostatin and leupeptin. Nonspecific binding was determined using 1 μM TCP. Incubations were terminated by filtration using a Brandell cell harvester. Whatman GF/B filters were presoaked in buffer containing 2% polyethylenimine. Each point was the mean of triplicate determinations, which differed by less than 10%. Time course experiments showed that equilibrium was achieved by 120 min at 0° in the absence or presence of either glutamate (100 μM) or AP7 (100 μM) and that these agents had no effect on binding at equilibrium. Protein was determined using the method of Lowry *et al.* (38).

Kinetic experiments. [^{3}H]TCP (5 nM) was incubated with membranes at 0° for 2 hr in the absence of drug (condition 1), in the presence of 1 μM (+)-MK801 (condition 2), or in the presence of 1 μM TCP (condition 3). The incubation was terminated by centrifugation of the membrane suspensions at 27,000 × *g* for 15 min. To initiate dissociation, the pellets were resuspended in the same volume of ice-cold 5 mM Tris·HCl, pH 8.2 buffer. The dissociation of [^{3}H]TCP was then monitored, as described for the [^{3}H]TCP assay, at 15-min intervals from 0 to 180 min. Because the time interval between resuspension of the pellets and filtration of the time 0 samples could not be precisely controlled for, a variable amount of ligand could have dissociated between the separate steps of centrifugation, resuspension, and filtration. Therefore, test drugs (Mg^{2+}, AP7, or glutamate) were added at the 30-min time point. (Note that the dissociation was not initiated by the addition of TCP.) The 15-min time point, therefore, occurred 15 min after the addition of agent or 45 min into the dissociation experiment. The percentage of control was calculated using the 30-min point as 100%. The 30-min time point (89.9 ± 4.7% of control) was barely different from control; thus, very little imprecision was introduced, because the dissociation was very slow. If the dissociation had been faster, then we would have run the risk of not observing a fast-dissociating component, but this was not the case (see Fig. 3). In other experiments, the resuspended membranes were added to test tubes prefilled with various agents. A time 0 point was determined by immediate filtration, whereas all other samples, including a condition without agents, were filtered 2.5 hr after the addition. The binding of

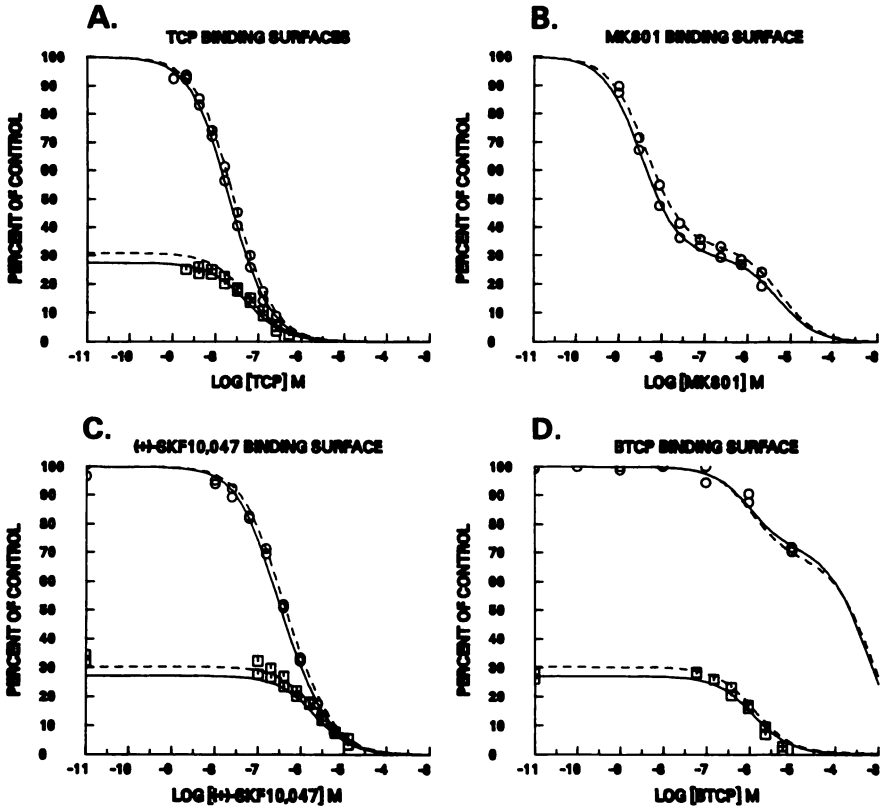

Fig. 1. Characterization of [^{3}H]TCP binding sites. TCP and (+)-MK801 binding surfaces. A, Displacement of 1.05 nM (——) and 5.35 nM (– – –) [^{3}H]TCP by eight concentrations of TCP in the absence (○) and presence (□) of 1 μM (+)-MK801. B, Displacement of 1.05 nM (——) and 5.35 nM (– – –) [^{3}H]TCP by eight concentrations of (+)-MK801. The specific binding at 1.05 and 5.35 nM [^{3}H]TCP was 85.8 and 349 fmol/mg of protein, respectively. Each *point* is the mean of three experiments, which differed by less than 10%. The entire set of data (54 points) were fit to a one-site binding model. The sum-of-squares was 1.00. Fitting the data to a two-site binding model resulted in a highly significant (*F* test; $p < 0.001$) reduction in the sum of squares to 0.069. The best-fit parameter estimates, reported in Table 1, generated the *lines* shown in the figure, illustrating the goodness of fit. (+)-SKF10.047 and BTCP binding surfaces. C, Displacement of 0.91 nM (——) and 4.64 nM (– – –) [^{3}H]TCP by concentrations of (+)-SKF10,047 in the absence (○) and presence (□) of 500 nM (+)-MK801. Each *point* is the mean of two experiments, which differed by less than 10%. The B_{max} and K_d values of TCP and (+)-MK801 were fixed to the values obtained from the data of A and B and the entire set of data (36 points) were fit to a one-site model (sum of squares = 0.133). Fitting the data to a two-site model resulted in a highly significant decrease in the sum of squares to 0.021 ($p < 0.001$, *F* test). The best-fit parameter values of the two-site model are reported in Table 1 and generated the lines in the figure. Similar experiments were conducted with PCP, (–)-cyclazocine, and etoxadrol. D, Displacement of 0.94 nM (——) and 4.29 nM (– – –) [^{3}H]TCP by concentrations of BTCP in the absence (○) and presence (□) of 500 nM (+)-MK801. The data were fit to a two-site model as described for C and the results are reported in Table 1.

TABLE 1

Best-fit parameter estimates of the two-site binding model

The TCP and (+)-MK801 binding surfaces depicted in Fig. 1 were fit to the two-site binding model for the best-fit parameter estimates (± standard error). As described in the legend to Fig. 1, the B_{max} and K_d values of TCP and (+)-MK801 were fixed to these values and the binding surfaces of the other drugs fit to the two-site binding model for the best-fit K_i values. These parameter values generated the lines in Fig. 1, visually illustrating the goodness-of-fit. Selectivity = K_d for site 2/K_d for site 1.

	Site 1	Site 2	Selectivity
B_{max} (fmol/mg of protein)	631 ± 10	829 ± 24	
TCP, K_d (nM)	14.1 ± 0.2	46.5 ± 1.3	3.3
(+)-MK801, K_i (nM)	3.22 ± 0.06	5208 ± 342	1617
Etoxadrol, K_i (nM)	21.5 ± 1.6	708 ± 77	32.9
PCP, K_i (nM)	58.3 ± 5.0	103 ± 14.7	1.9
(–)-Cyclazocine, K_i (nM)	99.7 ± 6.9	1990 ± 198	20.0
(+)-SKF10,047, K_i (nM)	180 ± 12	1816 ± 190	10.0
BTCP, K_i (nM)	$>10^6$	1083 ± 167	<0.001

[^{3}H]TCP to sites 1 and 2 were calculated as follows: site 1 = condition 1 – condition 2, and site 2 = condition 2 – condition 3. Data were fit (using MLAB) to a two-component dissociation model: $B/B_0 = A_1 \times e^{-K_1 t} + A_2 \times e^{-K_2 t}$, where B_0 is the binding observed at time 0, A_1 and A_2 are the percentage of specific binding bound to the two components, and K_1 and K_2 are the respective rate constants in units of min^{-1}.

Experiments using DA reuptake blockers. Guinea pig caudate membranes were prepared as described in Preparation of Membranes. Membranes were incubated for 60 min at 0°, in the absence (control) or presence of the indicated concentrations of GBR12909 or BTCP, in 55.2 mM sodium phosphate buffer, pH 7.4. The incubation was terminated by centrifugation (12,352 × *g*), and the membranes were washed twice by centrifugation in ice-cold 50 mM Tris·HCl, pH 8.2. The final pellets were resuspended in 5 mM Tris·HCl, pH 8.2, for assay with 5 nM [^{3}H]TCP in the absence or presence of 1 μM (+)-MK801.

Experimental design and analysis of equilibrium binding studies. These experiments were designed according to the method of binding surface analysis, which has been described in detail elsewhere (39, 40). Binding surfaces were generated by displacing two concentrations of [^{3}H]TCP (1 and 5 nM), each by eight concentrations of the test drug. Each displacement curve included determinations of total and nonspecific binding, yielding 10 data points (total, nonspecific, eight drug concentrations). The nonspecific binding was subtracted from each data point to yield the specific binding occurring in the absence and presence of test drugs. The specific binding in the presence of test drug was divided by the specific binding in the absence of test drug to yield a fraction of control. The data were pooled and fit to one- and two- site binding models. The two-site binding model is shown below:

$$G1S(L,LLI,J,K) = \frac{(B_1 \times L)}{\left(L + K_{D_1} \times \left(1 + \frac{LL}{K_{D_1}} + \frac{I}{K_{I_1}} + \frac{J}{K_{J_1}} + \frac{K}{K_{K_1}}\right)\right)} + \frac{(B_2 \times L)}{\left(L + K_{D_2} \times \left(1 + \frac{LL}{K_{D_2}} + \frac{I}{K_{I_2}} + \frac{J}{K_{J_2}} + \frac{K}{K_{K_2}}\right)\right)} \quad (1)$$

$$GD1(L,LL,I,J,K) = \frac{G1S(L,LL,I,J,K)}{G1S(L,0,0,0,0)} \quad (2)$$

The corresponding equations used to weight the points are (40):

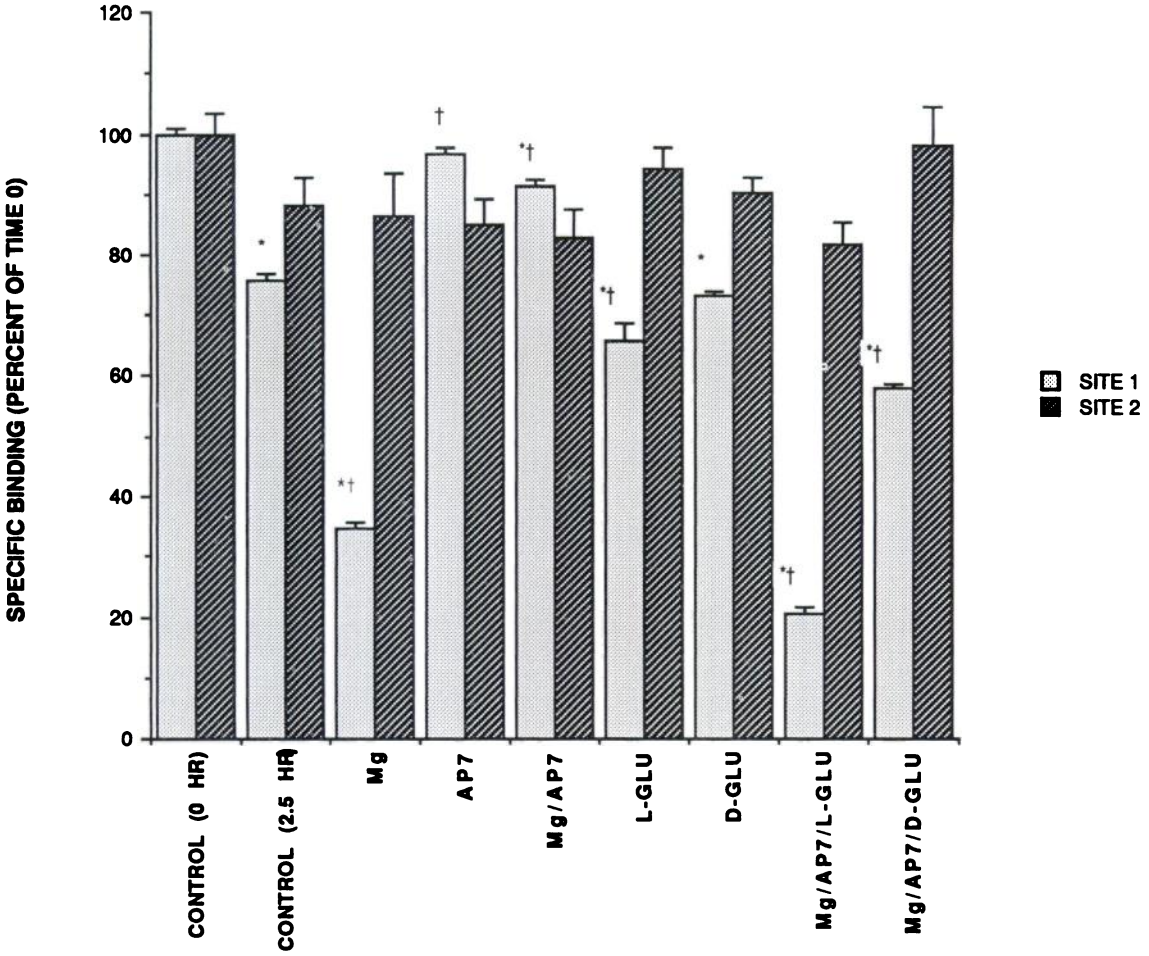

Fig. 2. Effect of agents on dissociation of [^{3}H]TCP. [^{3}H]TCP (5 nM) was incubated with membranes at 0° for 2 hr in the absence of drug (condition 1), in the presence of 1 μM (+)-MK801 (condition 2), and in the presence of 1 μM TCP (condition 3). The incubation was terminated by centrifugation at 27,000 × *g* for 15 min. To initiate dissociation, the pellets were resuspended in the same volume of ice-cold 5 mM Tris·HCl, pH 8.2, buffer. One-milliliter aliquots were distributed to test tubes prefilled with various agents (all at a final concentration of 100 μM). Control conditions received no agents. A time 0 point was filtered immediately. All other points were filtered 2.5 hr after the addition of membranes. Each value is the mean ± standard deviation of three determinations and are expressed as a percentage of the time 0 point. *$p < 0.01$ when compared with the time 0 point. †$p < 0.01$ when compared with the 2.5 hr control. The entire experiment was repeated with similar results (analysis of variance).

$$G1SW(L,LL,I,J,K) = \frac{1}{(0.0001 \times G1S(L,LL,I,J,K) \times G1S(L,LL,I,J,K))} \quad (1')$$

$$GD1W(L,LL,I,J,K) = \frac{1}{(0.0001 + 0.0003 \times GD1(L,LL,I,J,K))} \quad (2')$$

In these equations, L refers to the concentration of radiolabeled ligand, [^{3}H]TCP, LL to the concentration of nonradioactive ligand, TCP, I to inhibitor 1 ((+)-MK801), J to inhibitor 2, and K to inhibitor 3. K_{D_1}, K_{I_1}, K_{J_1}, and K_{K_1} are the equilibrium dissociation constants for L, I, J, and K for site 1. K_{D_2}, K_{I_2}, K_{J_2}, and K_{K_2} are the equilibrium dissociation constants for L, I, J, and K for site 2. Eq. 1 is a standard two-site competitive binding model, except that L and LL are constrained to have the same dissociation constant. Eq. 2 computes a "fraction of control" by dividing the binding observed in the presence of drugs by the binding in the presence of L alone.

To illustrate the application of these equations to the experimental design used in this study, consider the displacement of two concentrations of L, each by eight concentrations of LL. This surface requires two measurements of total binding, two measurements of nonspecific binding, and measurements of binding in the presence of the 16 different concentrations of LL. These data generate two displacement curves, each associated with a level of specific binding and eight displacement points. The two specific binding points are fit using Eq. 1 and weighted using Eq. 1′, whereas the displacement curves are fit using Eq. 2 and weighted using Eq. 2′. Both data sets (18 data points) are fit simultaneously, after the specific binding points are divided by a scale factor (equal to the estimated B_{max}) so that their units range between 0 and 1, the same as the displacement curve points. The computer language MLAB (41) was used to fit the data. This program uses a weighted nonlinear least squares algorithm to minimize the sum of squares. Statistical differences between binding models were tested using the F test (39). Statistical differences among experimental groups were tested using Student's t test.

Chemicals. [^{3}H]TCP (specific activity, 42.2 Ci/mmol) was purchased from New England Nuclear. (+)-MK801 (42) and etoxadrol (43) were synthesized in Dr. Rice's laboratory. (+)-SKF10,047 and (−)-cyclazocine were supplied by Dr. Jacobson. Chymostatin and leupeptin were obtained from Cambridge Research Biochemicals. Other reagents were supplied by Sigma Chemical Company. GBR12909, glutamate, and AP7 were purchased from Research Biochemicals Incorporated. BTCP was generously provided by Dr. James Woods at the Department of Pharmacology, University of Michigan Medical School (Ann Arbor, MI).

Results

Equilibrium binding studies. Fig. 1 shows the TCP and (+)-MK801 binding surfaces. Fig. 1A depicts the displacement

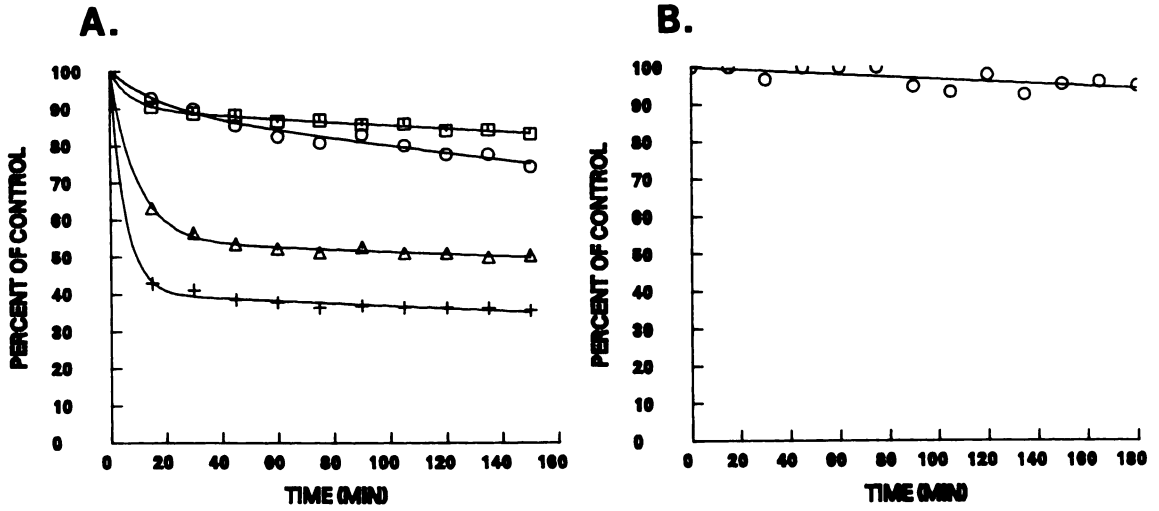

Fig. 3. Dissociation of [^{3}H]TCP from PCP site 1 and PCP site 2. Dissociation time courses were generated to examine the ability of agents (100 μM) to alter [^{3}H]TCP dissociation rates, as described in Materials and Methods. Each *point* is the mean of three independent experiments, which differed by less than 10%. A. Dissociation of [^{3}H]TCP from site 1. The dissociation of [^{3}H]TCP from site 1 was calculated as described in Materials and Methods. The data of the control (○), Mg^{2+} (△), Mg^{2+}/AP7 (□), and Mg^{2+}/AP7/glutamate (+) curves were not adequately described by a one-component dissociation model, so they were fit to a two-component dissociation model described in Materials and Methods. The best-fit parameter estimates reported in Table 2 were used to generate the *lines* in the figure. B. Dissociation of [^{3}H]TCP from site 2. The dissociation of [^{3}H]TCP from site 2 was calculated as described in Materials and Methods. The data of this condition were fit to a one-component dissociation model, with a resulting rate constant of 0.00033 ± 0.000054 min^{-1}. This parameter was used to draw the *line* in the figure.

TABLE 2

Effect of NMDA receptor modulators on PCP site 1

The data in Fig. 3A were fit to a two-component dissociation model for the best-fit parameter estimates reported below. Each value is ± standard deviation (three experiments).

	A_1	A_2	K_1	K_2
				min^{-1}
Control	10.2 ± 2.0	89.8 ± 1.8	0.052 ± 0.020	0.0012 ± 0.00015
Mg^{2+}	45.5 ± 1.6[a]	54.4 ± 1.2[a]	0.103 ± 0.010[a]	0.00060 ± 0.00022[a]
Mg^{2+}/AP7	10.2 ± 1.4	89.8 ± 1.0	0.13 ± 0.063[a]	0.00052 ± 0.00012[a]
Mg^{2+}/AP7/glutamate	59.6 ± 1.6[a,b]	40.3 ± 1.1[a,b]	0.19 ± 0.030[a]	0.00094 ± 0.00028[a,b]

[a] $p < 0.01$ when compared with control.
[b] $p < 0.01$ when compared with Mg^{2+}.

of two concentrations of [^{3}H]TCP by TCP, in the absence and presence of 1 μM (+)-MK801, thereby providing saturation binding information. Fig. 1B shows the (+)-MK801 binding surface. The concentration of (+)-MK801 used in Fig. 1A (1 μM) was chosen to "block" the binding of [^{3}H]TCP to the (+)-MK801-sensitive binding site, permitting more direct measurement of the (+)-MK801-insensitive binding site. Fitting the entire set of data (Fig. 1, A and B, 54 data points) to a one-site binding model resulted in a poor fit with a sum of squares of 1.00. Fitting the data to a two-site binding model resulted in a highly significant reduction in the sum of squares to 0.069 (F test; $p < 0.001$). The inhibitory dissociation constants of (−)-cyclazocine, PCP, and etoxadrol for the two binding sites were determined as described in Fig. 1, C and D.

The best-fit estimates of the K_d values of these drugs for the two binding sites, present at densities of 631 fmol/mg of protein (site 1) and 829 fmol/mg of protein (site 2), are reported in Table 1. PCP and its analog TCP bound with high affinity to both sites and were only slightly selective between them. In contrast, (+)-SKF10,047, (−)-cyclazocine, etoxadrol, and (+)-MK801 showed increasing degrees of selectivity for site 1. (+)-MK801 was about 1600-fold selective for site 1. BTCP, an analog of PCP that has a low affinity for rat brain PCP receptors, is a highly potent inhibitor of DA reuptake, (44) with a behavioral profile similar to that of cocaine (45). This agent had a K_i greater than 1 mM for site 1 and about 1 μM for site 2, making it about 1000-fold selective for site 2. Cocaine (10 μM) also inhibited [^{3}H]TCP binding to site 2 by 31%, without inhibiting binding to site 1 (data not shown).

Kinetic studies. The second prediction of the working hypothesis addressed by this study is that the (+)-MK801-sensitive binding site (site 1) is coupled to the NMDA receptor, whereas the (+)-MK801-insensitive binding site (site 2) is not. To assess coupling to the NMDA receptor, we conducted kinetic studies to examine the ability of glutamate (100 μM), an NMDA receptor agonist, AP7 (100 μM), a competitive NMDA receptor antagonist, and Mg^{2+} (100 μM), a noncompetitive NMDA receptor antagonist (18), to modulate the dissociation of [^{3}H]TCP from site 1 and site 2.

Membranes were incubated for 2 hr at 0° with 5 nM [^{3}H]TCP in three conditions: 1) total binding, 2) (+)-MK801 blocked (1 μM (+)-MK801), and 3) nonspecific binding (1 μM TCP). The incubation was terminated by centrifugation and the membranes of each condition were separately resuspended with ice-cold buffer. This design permitted the separate measurement of dissociation from site 1 (condition 1 − condition 2) and site 2 (condition 2 − condition 3). In the first set of experiments (Fig. 2), the homogenates (1-ml aliquots) were

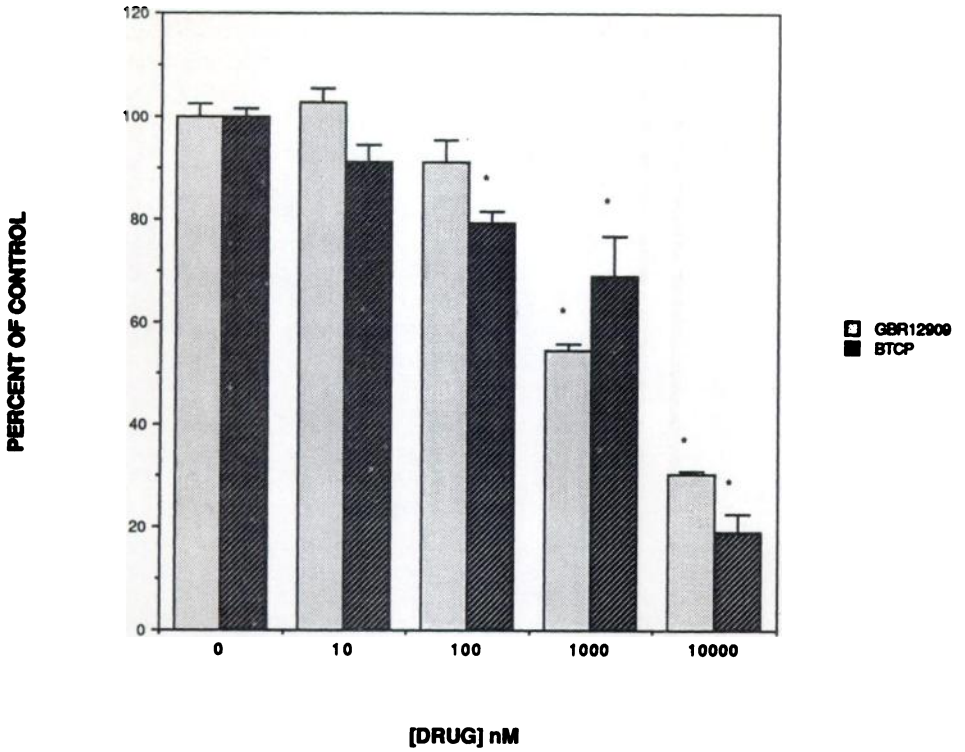

Fig. 4. Wash-resistant inhibition of [^{3}H]TCP binding to PCP site 2. Guinea pig caudate membranes were incubated for 60 min at 0° in 55.2 mM sodium phosphate buffer, pH 7.4, in the absence (control) and presence of the indicated concentrations of BTCP or GBR12909. The membranes were washed by centrifugation and [^{3}H]TCP binding sites were assayed as described in Materials and Methods. Neither drug had any significant effect on PCP site 1, except 10,000 nM BTCP, which inhibited [^{3}H]TCP binding to this site by 20%. *$p < 0.01$ when compared with control (Students t test).

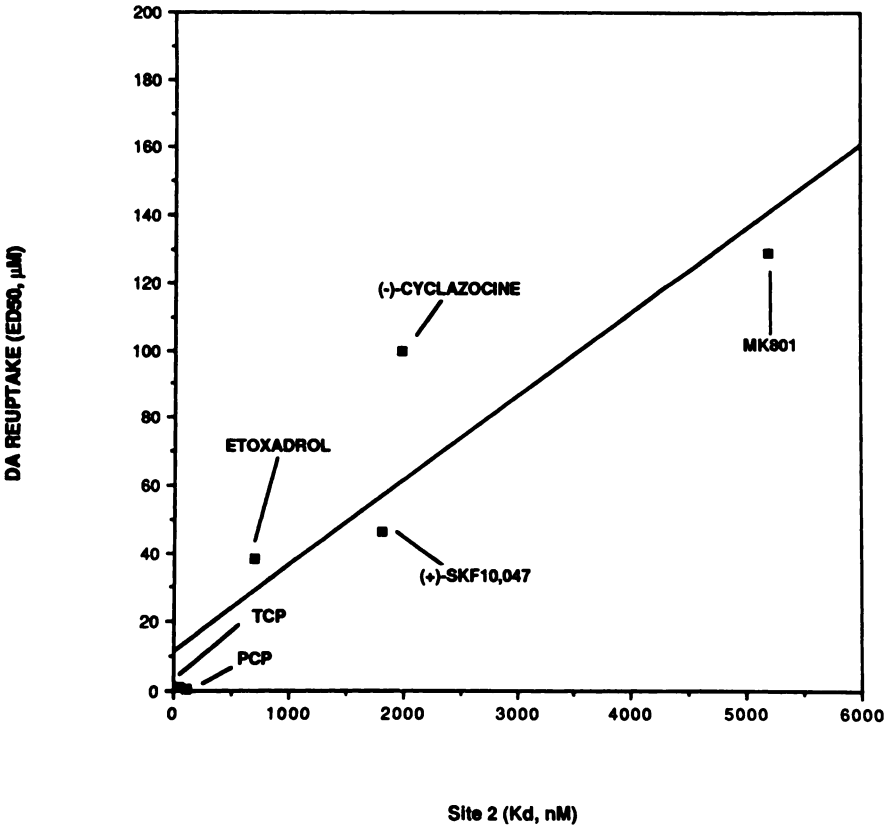

Fig. 5. Correlation of the K_i of agents for the (+)-MK801-insensitive binding site (site 2) with their ability to inhibit reuptake of [^{3}H]DA in striatal synaptosomes (23, 24).

either immediately filtered after resuspension (time 0) or added to test tubes prefilled with various agents and then filtered at 2.5 hr. In the second set of experiments (Fig. 3), the homogenates (1-ml aliquots) were filtered either immediately after resuspension (time 0) or at 15-min intervals thereafter. At the 30-min point, agents were added to the homogenates so as to determine their effect on the dissociation of [^{3}H]TCP from site 1 and site 2.

The dissociation of [^{3}H]TCP from site 1 and site 2 proceeded very slowly (Figs. 2 and 3). The experiment reported in Fig. 2 examined the ability of various agents to alter the rate at which [^{3}H]TCP dissociated from sites 1 and 2. The level of [^{3}H]TCP

binding to site 1 at the 2.5-hr time point was about 75% of control. In contrast, the level of [^{3}H]TCP binding to site 2 was not significantly different from that at time 0. No agent or combination of agents altered the dissociation of [^{3}H]TCP from site 2. Mg^{2+} (100 μM) markedly increased the dissociation of [^{3}H]TCP from site 1. AP7 (100 μM) slightly slowed and L-glutamate (100 μM) slightly increased the dissociation of [^{3}H]TCP from site 1. This effect was much more pronounced in the presence of Mg^{2+}. Thus, AP7 reversed the effect of Mg^{2+}, and L-glutamate reversed this effect of AP7. D-Glutamate (100 μM) had no effect when added alone but did partially reverse the Mg^{2+} dependent effect of AP7.

The experiments reported in Fig. 3 examined these observations in greater detail. Analysis of the dissociation data of site 1 (Fig. 3A) according to a two-component dissociation model (Table 2) resolved fast and slow components. Mg^{2+} doubled the fast rate constant (K_1), halved the slow rate constant (K_2), and increased the proportion of the fast component from 10.2% to 45.5%. The major effects of AP7 and glutamate were on the proportion of fast and slow dissociating components. The dissociation of [^{3}H]TCP from site 2 was very slow and was fit to a one-component dissociation model (see the legend to Fig. 3).

Experiments using DA reuptake blockers. To test the third prediction of the working hypothesis, membranes were preincubated with varying concentrations of the highly potent DA reuptake inhibitors BTCP and GBR12909 (46). As reported in Fig. 4, both drugs produced a selective, dose-dependent, and wash-resistant inhibition of [^{3}H]TCP binding to site 2. There was no effect on site 1, except for 10 μM BTCP, which caused a 20% wash-resistant inhibition.

Discussion

The working hypothesis addressed by this study, that only a portion of high affinity PCP receptors are coupled to the NMDA receptor, generated four predictions. The first, that quantitative ligand binding studies should resolve two high affinity binding sites, has been verified in this study.

Even though most laboratories, including our own (37), report only a single class of PCP binding sites in rat brain, several groups have observed two populations of PCP binding sites (47–50). The high affinity binding site identified by others is in all liklihood the same as PCP site 1. Although the present study used guinea pig brain and differed methodologically in several ways from other studies, one might hope to identify common characteristics among the various "low affinity" binding sites. Such characteristics might include anatomical distribution, ligand-selectivity pattern, and biochemical characteristics of binding sites. Unfortunately, gaps in the available data preclude a completely satisfactory comparison.

Mendelsohn *et al.* (47) reported a low affinity PCP binding site in rat cortical membranes that had a K_d of 7 μM and a B_{max} of 100 pmol/mg of protein. The binding parameters we observed for PCP site 2 in the present study are markedly different (Table 1). Moreover, it is possible that the low affinity site observed by Mendelsohn *et al.* (47) represents binding of [^{3}H]TCP to the haloperidol-sensitive σ receptor (51). The high affinity of PCP and TCP for sites 1 and 2 and the very low affinity of haloperidol and 1,3,-di(2-tolyl)guanidine for either site 1 or site 2 (>100 μM) in our study rule out the possibility that site 2 is the σ receptor (52).

Vignon *et al.* (48) reported in 1986 two [^{3}H]TCP binding sites. The higher affinity site (K_d = 5 to 10 nM) was enriched in rat forebrain, including cerebral cortex, whereas the lower affinity site (K_d = 50–80 nM) was enriched in the hindbrain and spinal cord. However, a subsequent study from the same group (50) reported an additional low affinity (K_d about 400 nM) [^{3}H]TCP binding site in rat cortex and cerebellum. They termed the high affinity site the TCP_A site, which is most likely the same as PCP site 1. The TCP_B site, which is found mainly in the cerebellum, is different from the low affinity site they report in the cortex, because the K_d of the cortical low affinity site is about 400 nM. It is possible that the TCP_B site corresponds to PCP site 2. Given the selective occurrence of the TCP_B site in the hind brain, one would expect the density of the TCP_A site to exceed that of the TCP_B site in whole brain homogenates. Contrary to that expectation, the density of PCP site 2 exceeds that of PCP site 1. However, their observation that (+)-MK801 displaced [^{3}H]TCP in a biphasic manner (50) is similar to our own and supports the hypothesis that their low affinity (K_d about 400 nM) site in the cortex might correspond to PCP site 2.

Haring *et al.* (49) also reported two PCP binding sites both by direct binding and by nonlinear least squares analysis of dexoxadrol displacement curves. Unlike the data of Chicheportiche *et al.* (50), who observed high and low affinity binding sites in rat cortex, Haring *et al.* (49) observed no detectable low affinity binding sites in rat cortex. Also, Haring *et al.* (49) did not examine the relative amounts of low and high affinity binding sites in the caudate, which is enriched with the DA reuptake complex. There are not sufficient ligand-selectivity data to permit a detailed comparison of their data with the data of the present study.

Itzhak (53, 54) has reported high and low affinity binding sites labeled by [^{3}H]3-OH-PCP, with K_d values of 0.45 and 18 nM and B_{max} values of 85 and 670 fmol/mg of protein. (+)-SKF10,047 is reported to have an IC_{50} of 61 nM at the high affinity site, which is calculated to be a K_i of 24 nM. Low affinity binding sites are labeled with 24 nM [^{3}H]3-OH-PCP in the presence of 100 nM (+)-SKF10,047, permitting a relatively selective assay of the low affinity site. The very low B_{max} of the high affinity site suggests that it is not the same as PCP site 1. Furthermore, pentazocine is reported to have an IC_{50} of 90 nM for the high affinity site, which is much lower than usually observed for high affinity PCP binding sites (37). In addition, cyclazocine and (+)-SKF10,047 have a much lower affinity for the low affinity [^{3}H]3-OH-PCP binding site than for [^{3}H]TCP binding sites (37).

Thus, although several groups have reported two classes of PCP binding sites, there is at present insufficient data to permit an unambiguous identification of PCP site 2 with these other low affinity binding sites. In a general sense, it seems that the TCP_B site has characteristics most similar to PCP site 2. Indeed, Chicheportiche *et al.* (50) postulated that the low affinity (K_d = 400 nM) site might in part include DA reuptake sites.

In this regard, it is interesting to note the high density of PCP site 2 in membranes prepared from whole guinea pig brain. In rats, the DA reuptake complex is highly localized to the caudate. If this is true for guinea pig brain as well, this would suggest that PCP site 2 might include binding sites other than the DA transporter. Our data that selective blockers of serotonin reuptake also cause a wash-resistant inhibition of

PCP site 2 support this concept.[2] Future studies will address this hypothesis.

Our ability to detect two high affinity binding sites for [^{3}H]TCP might reflect the quantitative methods used in this study. Several drugs examined in this study are only slightly selective for site 1. As described in detail by McGonigle *et al.* (56), the mathematically based approach to the design and analysis of ligand binding experiments used in this study (binding surface analysis) is required to accurately determine the K_d values of slightly selective drugs for two binding sites. Thus, it is likely, using a membrane preparation that possesses site 1 and site 2 in equal amounts or site 2 in low amounts, that the existence of two binding sites would not be detected unless the binding surface approach were used. Alternatively, it is equally likely that for reasons that remain enigmatic, although readily measured using guinea pig brain membranes, PCP site 2 is not detectable using rat brain membranes.

A direct prediction of our findings is that administration of PCP should produce effects (via PCP site 2) that are not produced by drugs such as (+)-MK801 or etoxadrol that are selective for site 1. Consistent with this, Pechnick *et al.* (57, 58) demonstrated that, whereas administration of PCP to rats causes a hypothermic response, dexoxadrol, which is also selective for site 1,[2] produces a hyperthermic response. Moreover, the effect of PCP was reversed by pretreatment of rats with metaphit, which acylates the DA reuptake complex (59).

The kinetic data strongly support the second prediction of the working hypothesis addressed by this study, that the (+)-MK801-sensitive binding site is associated with the NMDA excitatory amino acid receptor complex, because only site 1 was modulated by AP7, Mg^{2+}, and glutamate. These results, obtained using guinea pig membranes, differ somewhat from results obtained using rat brain (16) and are the subject of another paper. Nevertheless, the fact that AP7, Mg^{2+}, and glutamate failed to alter the dissociation of [^{3}H]TCP from site 2 supports the hypothesis that site 2 is not associated with the NMDA receptor. Moreover, the two binding sites are probably not two states of the NMDA-associated PCP receptor, because PCP had similar K_d values at both sites and the addition of Mg^{2+} selectively accelerated the dissociation of [^{3}H]TCP from site 1.

As reported in Table 1, BTCP binds selectively but weakly to PCP site 2. Similar results were obtained for GBR12909.[2] It is well known that the binding of reuptake inhibitors to the DA reuptake complex requires Na^+ (44) and that Na^+ inhibits the binding of [^{3}H]TCP (60). The apparent low affinity interaction of TCP with the DA reuptake complex as labeled by [^{3}H]BTCP and of BTCP with the reuptake complex as labeled by [^{3}H]TCP may reflect differential effects of Na^+ on the reuptake binding site; in the presence of Na^+, the reuptake binding site adopts a conformation that recognizes BTCP with high affinity but TCP with very low affinity, but, in the absence of Na^+, the reuptake binding site adopts a conformation that recognizes BTCP with low affinity and TCP with high affinity.

According to the hypothesis, preincubation of membranes with high affinity DA reuptake blockers in the presence of Na^+, which favors their binding to the reuptake complex, i.e., NaCl, should produce a wash-resistant inhibition of [^{3}H]TCP binding to site 2, the assay of which must be conducted in the absence of NaCl. Consistent with this hypothesis, both GBR12909 and BTCP produced a dose-dependent wash-resistant inhibition of PCP site 2 (Fig. 4). That is, while the reuptake complex is occupied by the reuptake blocker by virtue of its slow rate of dissociation, the binding of [^{3}H]TCP to PCP site 2 is also inhibited. Also consistent with this hypothesis is the observation that the PCP affinity ligand metaphit (61) irreversibly inhibits the binding of [^{3}H]GBR12935 to the DA reuptake complex (59). These data support the hypothesis that PCP site 2 is a component of the DA reuptake complex.

The third prediction of the working hypothesis derives experimental support from data published in the literature. Snell and Johnson (23, 24) have shown that the PCP receptor which inhibits the NMDA-stimulated release of [^{3}H]acetylcholine from striatal slices and that which mediates inhibition of the reuptake of [^{3}H]DA by striatal synaptosomes exhibit different structure-activity relationships. In particular, PCP, etoxadrol, (+)-SKF10.047, and (−)-cyclazocine all potently inhibited the NMDA-stimulated release of [^{3}H]acetylcholine, whereas etoxadrol, (+)-SKF10,047, (−)-cyclazocine, and (+)-MK801 were much less potent than PCP in inhibiting the reuptake of [^{3}H]DA. The structure-activity profiles for the PCP receptor ligands observed in these bioassays closely parallel those observed in our binding assay. The excellent correlation between the K_d values of these drugs for site 2 and their ED_{50} values for inhibiting DA reuptake is reported in Fig. 5. BTCP was excluded from the correlation graph because, although it is derived chemically from PCP, it has the behavioral and neurochemical profile of a cocaine-like drug (44, 45).

Although several studies have demonstrated that PCP and congeners interact with the DA reuptake carrier (20, 44, 62), these interactions occur in the micromolar concentration range. For example, TCP inhibits [^{3}H]BTCP binding with a K_i of 1.33 μM and [^{3}H]DA reuptake with an IC_{50} of 1.29 μM (44). PCP and related drugs inhibit NMDA receptor-mediated responses in the nanomolar range, apparently supporting the hypothesis that the interactions of PCP with the DA reuptake carrier might not be pharmacologically relevant to its psychotomimetic properties in humans.

However, our finding that PCP binds with high affinity to a binding site associated with the DA reuptake complex suggests that inhibition of DA reuptake by PCP might play a major role in producing its psychotomimetic effects. This hypothesis is consistent with data that increased release of DA is associated with the production of psychotic behavior (12) and with the observations that a variety of drugs abused by humans, including PCP (34), increase synaptic DA levels in the mesolimbic system of rats (63, 64). Moreover, doses of PCP, in rats, that inhibit NMDA-mediated effects such as postischemic neuronal damage (55) and convulsions (65) are similar to those that elevate extracellular levels of DA as measured by *in vivo* microdialysis.[1] Given the high affinity interaction of PCP with the DA reuptake carrier observed in this study, it is tempting to speculate that the psychotomimetic and addictive properties of PCP are mediated in part by site 2. This hypothesis is supported by observations that systemic administration of (+)-MK801 to rats does not increase DA release from the nucleus accumbens, as measured using microdialysis.[1] The implication of this hypothesis is that the therapeutic and psychotomimetic effects of PCP in humans might be separable. The chemical synthesis of selective agonists, antagonists, and affinity ligands for the two

[2] Unpublished data.

PCP binding sites should provide the required tools to explore this hypothesis in a variety of experimental paradigms.

References

1. Chen, G., C. R. Ensor, D. Russell, and B. Bohner. The pharmacology of 1-(1-phenylcyclohexyl)piperidine-HCl. *J. Pharmacol. Exp. Ther.* **127**:241–250 (1959).
2. Meyer, J. S., F. Griefenstein, and M. De Vault. A new drug causing sensory deprivation. *J. Nerv. Ment. Dis.* **129**:54–61 (1959).
3. Luby, E. D., C. D. Bertram, G. Rosenbaum, J. S. Gottlieb, and R. Kelley. Study of a new schizophrenomimetic drug, sernyl. *A. M. A. Arch. Neurol. Psychiatry* **81**:363–369 (1959).
4. Peterson, R. C., and R. C. Stillman. Phencyclidine: an overview. *Natl. Inst. Drug Abuse Res. Monogr. Ser.* **21**:1–17, (1977).
5. Vincent, J. P., B. Kartalovski, P. Geneste, J. M. Kamenka, and M. Lazdunski. Interaction of phencyclidine ("angel dust") with a specific receptor in rat brain membranes. *Proc. Natl. Acad. Sci. USA* **76**:4678–4682 (1979).
6. Zukin, S. R., and R. S. Zukin. Specific ^{3}H-phencyclidine binding in rat central nervous system. *Proc. Natl. Acad. Sci. USA* **76**:5372–5376 (1979).
7. Brady, K. T., and R. L. Balster. Discriminative stimulus properties of phencyclidine and five analogues in the squirrel monkey. *Pharmacol. Biochem. Behav.* **14**:213–280 (1980).
8. Holtzman, S. G. Phencyclidine-like discriminative effects of opioids in the rat. *J. Pharmacol. Exp. Ther.* **214**:614–619 (1980).
9. Shannon, H. E. Evaluation of phencyclidine analogs on the basis of their discriminative stimulus properties in the rat. *J. Pharmacol. Exp. Ther.* **216**:543–551 (1981).
10. Haertzen, C. A. Subjective effects of narcotic antagonists cyclazocine and nalorphine on the addition research center inventory. *Psychopharmacologia* **18**:366–377 (1970).
11. Johnson, K. M. Phencyclidine: behavioral and biochemical evidence supporting a role for dopamine. *Fed. Proc.* **42**:2579–2583 (1983).
12. Meltzer, H. Y., and S. M. Stahl. The dopamine hypothesis of schizophrenia: a review. *Schizophr. Bull.* **2**:19–76 (1988).
13. Allen, R. M., and S. J. Young. Phencyclidine-induced psychosis. *Am. J. Psychiatry* **135**:1081–1084 (1978).
14. Domino, E. F., and E. D. Luby. Abnormal mental states induced by phencyclidine as a model for schizophrenia, in *PCP (Phencyclidine): Historical and Current Perspectives* (E. F. Domino, ed.). NPP Books, Ann Arbor, MI, 401–418 (1981).
15. Anis, N. A., S. C. Berry, N. R. Burton, and D. Lodge. The dissociative anaesthetics, ketamine and phencyclidine, selectively reduce excitation of central mammalian neurons by *N*-methyl-D-aspartate. *Br. J. Pharmacol.* **79**:565–575 (1983).
16. Loo, P. S., A. F. Braunwalder, J. Lehmann, M. Williams, and M. A. Sills. Interaction of L-glutamate and magnesium with phencyclidine recognition sites in rat brain: evidence for multiple affinity states of the phencyclidine/*N*-methyl-D-aspartate receptor complex. *Mol. Pharmacol.* **32**:820–830 (1987).
17. Javitt, D. C., A. Jotkowitz, R. Sircar, and S. R. Zukin. Non-competitive regulation of phencyclidine/*sigma*-receptors by the *N*-methyl-D-aspartate receptor antagonist D-(−)-2-amino-5-phosphonovaleric acid. *Neurosci. Lett.* **78**:193–198 (1987).
18. Cotman, C. W., and L. L. Iverson. Excitatory amino acids in the brain: focus on NMDA receptors. *Trends Neurosci.* **10**:263–265 (1987).
19. Bartschat, D. K., and M. P. Blaustein. Phencyclidine in low doses selectively blocks a presynaptic voltage-regulated potassium channel in rat brain. *Proc. Natl. Acad. Sci. USA* **83**:189–192 (1986).
20. Vignon, J., and M. Lazdunski. Structure-function relationships in the inhibition of synaptosomal dopamine uptake by phencyclidine and analogues: potential correlation with binding site identified with [^{3}H]phencyclidine. *Biochem. Pharmacol.* **33**:700–702 (1984).
21. Tam, S. W., and L. Cook. *Sigma* opiates and certain antipsychotic drugs mutually inhibit (+)-[^{3}H]SKF10,047 and [^{3}H]haloperidol binding in guinea pig brain membranes. *Proc. Natl. Acad. Sci. USA* **81**:5618–5621 (1984).
22. Albuquerque, E. X., M. C. Tsai, R. S. Aronstam, B. Witcop, A. T. Eldefrawi, and M. E. Eldefrawi. Phencyclidine interactions with the ionic channel of the acetylcholine receptor and electrogenic membrane. *Proc. Natl. Acad. Sci. USA* **77**:1224–1228 (1980).
23. Johnson, K. M., and L. D. Snell. Effects of phencyclidine (PCP)-like drugs on turning behavior, ^{3}H-dopamine uptake, and ^{3}H-PCP binding. *Pharmacol. Biochem. Behav.* **22**:731–735 (1985).
24. Snell, L. D., and K. M. Johnson. Antagonism of *N*-methyl-D-aspartate-induced transmitter release in the rat striatum by phencyclidine-like drugs and its relationship to turning behavior. *J. Pharmacol. Exp. Ther.* **235**:50–57 (1985).
25. Jarvis, M. F., D. E. Murphy, and M. Williams. Quantitative autoradiographic localization of NMDA receptors in rat brain using [^{3}H]CPP: comparison with [^{3}H]TCP binding sites. *Eur. J. Pharmacol.* **141**:149–152 (1987).
26. Snell, L. D., S. J. Yi, and K. M. Johnson. Comparisons of the effects of MK801 and phencyclidine on catecholamine uptake and NMDA-induced norepinephrine release. *Eur. J. Pharmacol.* **145**:223–226 (1988).
27. Koek, W., and J. H. Woods. Assessing directly observable behavioral effects of phencyclidine (PCP)-like drugs in pigeons, in rats, and in rhesus monkeys, in *Sigma and Phencyclidine-Like Compounds as Molecular Probes in Biology* (E. F. Domino and J. M. Kamenka, eds.). NPP Books, Ann Arbor, MI, 493–509 (1988).
28. Koek, W., and J. H. Woods. Correlations between phencyclidine-like activity and *N*-methyl-D-aspartate antagonism: behavioral evidence, in *Sigma and Phencyclidine- Like Compounds as Molecular Probes in Biology* (E. F. Domino, and J. M. Kamenka, eds.). NPP Books, Ann Arbor, MI, 357–372 (1988).
29. Sircar, R., M. Rappaport, R. Nichtenhauser, and S. R. Zukin. The novel anticonvulsant MK801: a potent and specific ligand of the brain phencyclidine/*sigma*-receptor. *Brain Res.* **435**:235–240 (1987).
30. Willets, J., and R. L. Balster. Phencyclidine-like discriminative stimulus properties of MK801 in rats. *Eur. J. Pharmacol.* **146**:167–169 (1988).
31. Tricklebank, M. D., L. Singh, R. J. Oles, E. H. Wong, and S. D. Iversen. A role for receptors of *N*-methyl-D-aspartate acid in the discriminative stimulus properties of phencyclidine. *Eur. J. Pharmacol.* **141**:497–501 (1987).
32. Jackson, A., and D. J. Sanger. Is the discriminative stimulus produced by phencyclidine due to an interaction with *N*-methyl-D-aspartate receptors? *Psychopharmacology* **96**:87–92 (1988).
33. Willetts, J., and R. L. Balster. The discriminative stimulus effects of *N*-methyl-D-aspartate antagonists in phencyclidine-trained rats. *Neuropharmacology* **27**:1249–1256 (1988).
34. Hernandez, L., F. Lee, and B. G. Hoebel. Microdialysis in the nucleus accumbens during feeding of drugs of abuse: amphetamine, cocaine and phencyclidine. *Ann. N. Y. Acad. Sci.* **537**:508–511 (1988).
35. Troupin, A. S., J. R. Mendius, F. Cheng, and M. W. Risinger. MK801 in *Current Problems in Epilepsy: New Anticonvulsant Drugs* (B. S. Meldrum and R. J. Porter, eds.). Libbey, London, 191–202 (1986).
36. Rice, K. C., M. M. Mattson, A. E. Jacobson, J. A. Monn, A. A. Reid, and R. B. Rothman. The phencyclidine analog ^{3}H-TCP labels two binding sites in guinea pig brain distinguished by (+)-MK801. *Neurosci. Abstr.* **199.2** (1988).
37. Rothman, R. B., V. Bykov, A.-H. Newman, A. E. Jacobson, and K. C. Rice. Interaction of enantiomeric pairs of opiates with phencyclidine binding sites in rat brain: identification of (+)-pentazocine as a ligand potentially suitable for imaging *sigma* binding sites using positron emission tomography. *Neuropeptides* **12**:1–5 (1988).
38. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265–275 (1951).
39. Rothman, R. B. Binding surface analysis: an intuitive yet quantitative method for the design and analysis of ligand binding studies. *Alcohol Drug Res.* **6**:309–325 (1986).
40. Rothman, R. B., J. B. Long, V. Bykov, A. E. Jacobson, K. C. Rice, and J. W. Holaday. *Beta*-FNA binds irreversibly to the opiate receptor complex: *in vivo* and *in vitro* evidence. *J. Pharmacol. Exp. Ther.* **247**:405–416 (1988).
41. Knott, G. D., and D. K. Reece. MLAB: a civilized curve fitting system. *Proc. Online 1972 Int. Conf.* **1**:497–526 (1972).
42. Hardie, W. R., J. Hidalgo, I. F. Halverstadt, and R. E. Allen. 4-(2-Piperidyl)-1,3-dioxolanes with local anesthetic, spasmolytic and central nervous system activity. *J. Med. Chem.* **9**:127–136 (1966).
43. Lamanec, T. R., D. R. Bender, A. M. DeMarco, S. Karady, R. A. Reamer, and L. M. Weinstock. *Alpha* effect nucleophiles: a novel and convenient method for the synthesis of dibenz[*a*,*d*]cycloheptenimines. *J. Org. Chem.* **53**:1768–1774 (1988).
44. Vignon, J., V. Pinet, C. Cerruti, J.-M. Kamenka, and R. Chicheportiche. [^{3}H]-[1-(2-Benzo(*b*)thiophenyl)cyclohexyl]piperidine ([^{3}H]BTCP): a new phencyclidine analog selective for the dopamine uptake complex. *Eur. J. Pharmacol.* **148**:427–436 (1988).
45. Koek, W., G. Winger, F. C. Colpaert, J. W. Woods, and J. M. Kamenka. The PCP analog BTCP shares cocaine-like but not other characteristic behavioral effects with PCP and MK801. *Pharmacologist* (1989).
46. Heikkala, R. E., and L. Manzino. Behavioral properties of GBR 12909, GBR 13069 and GBR 13098: specific inhibitors of dopamine uptake. *Eur. J. Pharmacol.* **103**:241–248 (1984).
47. Mendelsohn, L. G., G. A. Kerchner, V. Kalra, D. M. Zimmerman, and J. D. Leander. Phencyclidine receptors in rat brain cortex. *Biochem. Pharmacol.* **33**:3529–3535 (1984).
48. Vignon, J., A. Privat, I. Chaudieu, A. Thierry, J.-M. Kamenka, and R. Chicheportiche. [^{3}H]Thienyl-phencyclidine ([^{3}H]TCP) binds to two different sites in rat brain: localization by autoradiographic and biochemical techniques. *Brain Res.* **378**:133–141 (1986).
49. Haring, R., Y. Kloog, A. Kalir, and M. Sokolovsky. Binding studies and photoaffinity labeling identify two classes of phencyclidine receptors in rat brain. *Biochemistry* **26**:5854–5861 (1987).
50. Chicheportiche, R., Y. Agid, I. Chaudieu, *et al.* The multiple binding sites of [^{3}H]PCP and [^{3}H]TCP in the rat and human CNS, in *Sigma and Phencyclidine-Like Compounds as Molecular Probes in Biology* (E. F. Domino, and J. M. Kamenka, eds.). NPP Books, Ann Arbor, MI, 107–116 (1988).
51. Sonders, M. S., J. F. W. Keana, and E. Weber. Phencyclidine and psychotomimetic *sigma* opiates: recent insights into their biochemical and physiological sites of action. *Trends Neurosci.* **11**:37–40 (1988).
52. Rice, K. C., M. V. Mattson, A. E. Jacobson, J. A. Monn, A. A. Reid, and R. B. Rothman. The phencyclidine analog [^{3}H]TCP labels two binding sites

in guinea pig brain distinguished by (+)-MK801. *Soc. Neurosci. Abstr.* **482** (1988).
53. Itzhak, Y. Pharmacological specificity of some psychotomimetic and antipsychotic agents for the *sigma* and PCP binding sites. *Life. Sci.* **42**:745–752 (1988).
54. Itzhak, Y. [^{3}H]PCP-3-OH and (+)-[^{3}H]SKF10047 bind to multiple *sigma*/opiate/PCP binding sites in rat brain, in *Sigma and Phencyclidine-Like Compounds as Molecular Probes in Biology* (E. F. Domino, and J. M. Kamenka, eds.). NPP Books, Ann Arbor, MI, 129–138 (1988).
55. Sauer, D., J. Nuglisch, C. Rossberg, H. D. Mennel, T. Beck, G. W. Bielenberg, and J. Krieglstein. Phencyclidine reduces postischemic neuronal necrosis in rat hippocampus without changing blood flow. *Neurosci. Lett.* **91**:327–332 (1988).
56. McGonigle, P., K. A. Neve, and P. B. Molinoff. A quantitative method of analyzing the interaction of slightly selective radioligands with multiple receptor subtypes. *Mol. Pharmacol.* **30**:329–337 (1986).
57. Pechnick, R. N., C. A. Wong, R. George, A. Thurkauf, A. E. Jacobson, and K. C. Rice. Comparison of the acute effects of dexoxadrol, levoxadrol, and phencyclidine on body temperature in the rat. *Neuropharmacology,* **28**:829–835 (1989).
58. Pechnick, R. N., A. A. Wu, B. M. Chun, R. George, A. Thurkauf, A. E. Jacobson, and K. C. Rice. Metaphit antagonizes phencyclidine-induced hypothermia in the rat. *Life Sci.*, **45**:439–445 (1989).
59. Berger, P., A. E. Jacobsen, K. C. Rice, R. A. Lessor, and M. A. Reith. Metaphit, a receptor acylator, inactivates cocaine binding sites in striatum and antagonizes cocaine-induced locomotor stimulation in rodents. *Neuropharmacology* **25**:931–933 (1986).
60. Vincent, J. P., J. N. Bidard, M. Lazdunski, G. Romey, Y. Tourneur, and J. Vignon. Identification and properties of phencyclidine-binding sites in nervous tissues. *Fed. Proc.* **42**:2570–2573 (1983).
61. Rafferty, M. F., M. Mattson, A. E. Jacobson, and K. C. Rice. A specific acylating agent for the [^{3}H]phencyclidine receptors in rat brain. *FEBS Lett.* **181**:318–322 (1985).
62. Schweri, M. M., P. Skolnick, M. F. Rafferty, K. C. Rice, A. J. Janowsky, and S. M. Paul. [^{3}H]Threo-(±)-methylphenidate binding to 3,4-dihydroxyphenylethylamine uptake sites in corpus striatum: correlation with the stimulant properties of ritalinic acid esters. *J. Neurochem.* **45**:1062–1070 (1985).
63. Ritz, M. C., R. J. Lamb, S. R. Goldberg, and M. J. Kuhar. Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science (Wash. D. C.)* **237**:1219–1223 (1987).
64. Chiara, G. D., and A. Imperato. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc. Natl. Acad. Sci. USA* **85**:5274–5278 (1988).
65. Leander, J. D., R. C. Rathbun, and D. M. Zimmerman. Anticonvulsant effects of phencyclidine-like drugs: relation to *N*-methyl-D-aspartate acid antagonism. *Brain Res.* **454**:368–372 (1988).

Send reprint requests to: Richard B. Rothman, Unit on Receptor Studies, LCS, NIMH, Bldg. 10-3D41, Bethesda, MD 20892.