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# Tricyclic Antidepressants and Dextromethorphan Bind with Higher Affinity to the Phencyclidine Receptor in the Absence of Magnesium and L-Glutamate

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**SUMMARY**

Recent studies from our laboratory have provided evidence that multiple states of the phencyclidine (PCP) receptor exist. In addition, several compounds such as PCP and the novel anticonvulsant MK-801 were found to inhibit binding more potently in the presence of $Mg^{2+}$ and L-glutamate (L-GLU) than when these agents were excluded from the binding assay. In the present study, a number of pharmacological compounds that have been suggested to interact within the *N*-methyl-D-aspartate (NMDA) receptor complex, including tricyclic antidepressants (TCAs), were examined for their ability to inhibit the binding of [$^3$H]1-[1-(2-thienyl)cyclohexyl]piperidine ([$^3$H]TCP) in the absence or presence of $Mg^{2+}$ and L-GLU. The TCAs imipramine, amitriptyline, and opipramol produced shallow inhibition curves in the absence of $Mg^{2+}$ and L-GLU. Computer analysis of the binding data indicated that a two-component binding model described the data significantly better than a one-component model. In the presence of $Mg^{2+}$ and L-GLU, the inhibition curves became steeper and were shifted to the right, and computer analysis of the binding data indicated that a one-component model adequately described the binding data. A series of other centrally active compounds, including several antipsychotics and antihistamines, the antiparkinsonian anticholinergic trihexyphenidyl and the antitussive dextromethorphan, were also found to be affected similarly by the inclusion of $Mg^{2+}$ and L-GLU in the binding assay. Dextrorphan, in contrast to dextromethorphan, inhibited [$^3$H]TCP binding more potently in the presence of $Mg^{2+}$ and L-GLU. The present results suggest that the compounds that inhibit binding more potently in the absence of $Mg^{2+}$ and L-GLU are interacting with the PCP receptor in a different manner from that of PCP and MK-801, because these open-channel blockers inhibit [$^3$H]TCP binding more potently in the presence of $Mg^{2+}$ and L-GLU. The data support previous findings that TCAs interact with the NMDA receptor complex and suggest that the compounds trihexyphenidyl and dextromethorphan, which have been shown to block NMDA-mediated neurotoxicity, may produce their effects through an interaction with the PCP receptor, albeit by a different mechanism from that of open-channel blockers.

The NMDA receptor and its associated complex is the subject of considerable research effort at the present time and has provided new insights into potential mechanisms of ischemia-related neurodegeneration (1, 2) and anticonvulsant actions (3). The receptor complex is currently thought to be composed of the NMDA, PCP, and strychnine-insensitive glycine receptors, a magnesium site, a zinc site, and an ion channel (4–13).

Dissociative anesthetics such as PCP and dexoxadrol and the novel anticonvulsant MK-801 (14) noncompetitively inhibit NMDA-induced effects (15), most likely by binding to the PCP receptor within the ion channel and blocking conduction of ions through the channel (16). These compounds have been termed open-channel blockers (16, 17).

Conversely, compounds acting at the NMDA receptor modulate the PCP receptor. NMDA agonists such as L-GLU induce or stabilize a high affinity state of the PCP receptor, whereas NMDA antagonists such as CPP eliminate or destabilize this high affinity state of the PCP receptor (18).

Previously, the dissociative anesthetics and MK-801 were found to generate shallow inhibition curves for the binding of [$^3$H]TCP in the absence of $Mg^{2+}$ and L-GLU. Computer analysis indicated two components of binding existed under these conditions. In contrast, only one binding component was found when $Mg^{2+}$ and L-GLU were included in the binding assay, and the inhibition curves for these compounds shifted to the left. In the present study, a series of centrally active compounds including TCAs, certain antipsychotics, and antihistamines, as well as the antiparkinsonian anticholinergic compound trihexyphenidyl, the antitussive antiischemic dextromethorphan (19–21), and the analgesic antiischemic dextrorphan (19, 22), were examined for their ability to inhibit the binding of [$^3$H]TCP in the absence and presence of $Mg^{2+}$ and L-GLU. The

**ABBREVIATIONS:** NMDA, *N*-methyl-D-aspartate; CPP, 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid; DTG, 1,3-di-*o*-tolylguanidine; D-EKC, D-ethylketocyclazocine; GABA, γ-aminobutyric acid; MK-801, (+)-4-methyl-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5,10-imine; PCP, phencyclidine; TCA, tricyclic antidepressant; TCP, 1-[1-(2-thienyl)cyclohexyl]piperidine; L-GLU, L-glutamate; G protein, guanine nucleotide-binding protein.

results indicate that compounds exist that, in contrast to the dissociative anesthetics and MK-801, inhibit [$^3$H]TCP binding more potently in the absence of $Mg^{2+}$ and L-GLU. The significance of these findings is discussed within the framework of the NMDA receptor complex and the pharmacological effects of the compounds studied.

## Materials and Methods

**Membrane preparation.** Rat forebrain membranes were prepared as described previously (18). Forebrains from male Sprague-Dawley rats [150–250 g; Marland Farms, Mbf:(SD)] were homogenized in 50 volumes original tissue weight of 5 mM Tris·HCl buffer (pH 7.7 at 25°), using a Brinkmann Polytron, setting 6, for 25 sec. Homogenates were centrifuged for 10 min at 48,000 × *g* (Beckman J2-21M). To remove endogenous excitatory amino acids and divalent cations, pellets were resuspended in 50 volumes of 5 mM Tris-HCl containing 10 mM disodium EDTA, incubated at 37° for 10 min, and recentrifuged. Pellets were washed again by resuspension and centrifugation. The pellet from this second wash was resuspended in 50 volumes of 5 mM Tris·HCl and was stored in suspension at −20° for at least 5 days. On the day of the experiment, the suspension was thawed, centrifuged, and washed an additional two times. The final pellet was resuspended in 125 volumes original tissue weight of 5 mM Tris·HCl buffer, pH 7.7 (0.2 mg of protein/ml).

**Receptor binding.** Binding assays were conducted as described previously (18). Briefly, binding assays were initiated by the addition of 1.0 ml of membrane suspension to test tubes that contained 500 μl of buffer, 5 nM [$^3$H]TCP (100 μl; NEN-Dupont, Boston, MA), either 30 μM $MgCl_2$ (100 μl) and 0.5 μM L-glutamic acid (100 μl) or 200 μl of buffer, and 200 μl of drug or buffer. In the competition experiments, 10–12 different concentrations of competitor were incubated with 5 nM [$^3$H]TCP. All assays were performed in triplicate.

The binding reaction was carried out at 25° for 2 hr. The reaction was terminated by filtration under reduced pressure through Whatman GF/B filters that were presoaked in 0.05% polyethylenimine. The filters were rapidly washed with three 6.5-ml aliquots of buffer. The filters were equilibrated in 3.5 ml of the scintillant Formula-989 (Dupont-NEN) and radioactivity bound to the filter was measured by liquid scintillation counting at an efficiency of 45 to 55%. Specific binding of [$^3$H]TCP was defined as total binding minus the binding in the presence of 100 μM dexoxadrol or PCP.

**Drugs.** Drugs were generously donated by the following companies: atropine sulfate and benztropine mesylate (Merck, Sharp and Dohme Research Laboratories, West Point, PA); chlorpromazine and trifluoperazine HCl (Smith Kline and French Laboratories, Philadelphia, PA); chlorprothixene, dextrorphan tartrate, and diazepam (Hoffman-La Roche, Nutley, NJ); clozapine and methysergide (Sandoz Pharmaceuticals, Hanover, NJ); D-EKC (Sterling-Winthrop Research Institute, Rensselaer, NY); dilantin sodium (Parke-Davis, Ann Arbor, MI); fluoxetine HCl (Eli Lilly and Co., Indianapolis, IN); metrazol (Knoll Pharmaceutical Co., Whippany, NJ); naloxone (Endo Laboratories, Garden City, NY); phenobarbital sodium (Winthrop, New York, NY); trihexyphenidyl (Lederle Laboratories, Pearl River, NY); and verapamil (Pfizer, Inc., New York, NY). Opipramol HCl, spiperone, and sulpiride were synthesized in the Chemistry Department, CIBA-GEIGY (Summit, NJ). DTG was purchased from Aldrich Chemical Co. (Milwaukee, WI). All other drugs were purchased from Sigma Chemical Co. (St. Louis, MO).

**Computer analysis of binding data.** The results from replicate binding experiments were analyzed by nonlinear regression analysis using RS/1 (Bolt, Beranek and Newman, Boston, MA) on a VAX 8800 mainframe. Whether a two-component binding model fit the data significantly better than a one-component model was determined using the partial *F* test ($p < 0.01$). A three-component binding model was not found in any experiment to fit the data significantly better than a two-component model. The results shown are represented as the mean ± standard error generated by this analysis.

## Results

Previously, the open-channel blockers PCP and MK-801 were found to inhibit the binding of [$^3$H]TCP more potently in the presence of $Mg^{2+}$ and L-GLU than in their absence (18). These data supported the findings that multiple states of the PCP receptor exist. Since that time, a number of compounds from other therapeutic classes have been suggested to interact with the NMDA receptor complex. For example, recent studies have suggested that TCAs interact with the zinc component of the NMDA receptor complex (23). To further study their interaction with the PCP receptor, several TCAs were examined for their ability to inhibit the binding of [$^3$H]TCP in the absence and presence of $Mg^{2+}$ and L-GLU. In addition, several antipsychotic and antihistamine compounds with structural similarities to the TCAs were also examined. These results are summarized in Table 1. Essentially, all 11 compounds studied produced similar binding profiles. The inhibition curves generated by the TCA imipramine, the antipsychotic trifluoperazine, and the antihistamine pyrilamine (Figs. 1–3) are shown to represent each pharmacological class. Except for desipramine, all compounds produced shallow inhibition curves of the binding of [$^3$H]TCP in the absence of $Mg^{2+}$ and L-GLU (basal conditions), with Hill coefficients significantly less than 1. Computer analysis revealed that a two-component binding model described the data significantly better than a one-component model. The $IC_{50}$ values for the high affinity component ranged from 111 nM for opipramol to 2000 nM for desipramine. In contrast to PCP and MK-801, the high affinity component of the inhibition curves was eliminated when $Mg^{2+}$ and L-GLU were included in the binding assay, with the result that the competition curves steepened and were shifted to the right (Figs. 1–3). As reflected by the increase in the Hill coefficient (Table 1), a one-component binding model now adequately described the binding data.

For the antidepressant desipramine, a one-component binding model adequately described the data under basal conditions, although the Hill coefficient was increased from 0.72 to 0.99 when $Mg^{2+}$ and L-GLU were included in the binding assay. Furthermore, this compound was also more potent in inhibiting [$^3$H]TCP binding in the absence of $Mg^{2+}$ and L-GLU than in the presence of these agents, as shown by the increase in the $IC_{50}$ value from 2000 to 8000 nM.

Based on the recent finding that dextromethorphan, dextrorphan, and trihexyphenidyl can block glutamate-induced neurotoxicity (24, 25), the ability of these compounds and benztropine, another antiparkinsonian compound, to inhibit [$^3$H]TCP binding were examined. Dextromethorphan (Fig. 4), benztropine, and trihexyphenidyl generated competition curves similar to those produced by the TCAs. Shallow inhibition curves were generated in the absence of $Mg^{2+}$ and L-GLU. Dextromethorphan displayed one of the lowest $IC_{50}$ values, 34 nM, for the high affinity binding component (Table 1). When $Mg^{2+}$ and L-GLU were included in the binding assay, the competition curves for these three compounds steepended and were shifted to the right.

Dextrorphan, however, yielded a different binding profile than dextromethorphan (Table 1). Although a shallow inhibition curve was generated under basal conditions, the competi-

TABLE 1

**Effect of $Mg^{2+}$ and L-GLU on inhibition curves of [$^3$H]TCP binding**

The values represent the binding parameters obtained from computer analysis for the high and low affinity components of the inhibition curve obtained for each compound. The following compounds inhibited less than 20% of specific binding at a concentration of 10 μM: atropine sulfate, carbamazepine, clozapine, dilantin, D-EKC, diazepam, fluoxetine, GABA, methysergide, metrazol, naloxone, phenobarbital, sulpiride, and verapamil.

| Compound | High Affinity | | Low Affinity | | $n_H$ |
|---|---|---|---|---|---|
| | Bound | $IC_{50}$ | Bound | $IC_{50}$ | |
| | % | nM | % | nM | |
| Amitriptyline | | | | | |
| −Mg/GLU | 45 ± 7 | 29 ± 9 | 56 ± 7 | 8,000 ± 2,000 | 0.69 ± 0.03 |
| +Mg/GLU | | | 102 ± 1 | 20,000 ± 1,000 | 1.63 ± 0.04 |
| Benztropine | | | | | |
| −Mg/GLU | 42 ± 2 | 120 ± 30 | 58 ± 2 | 100,000 ± 17,000 | 0.34 ± 0.01 |
| +Mg/GLU | | | 100 ± 1 | 138,000 ± 5,000 | 1.5 ± 0.07 |
| Chlorpromazine | | | | | |
| −Mg/GLU | 24 ± 8 | 120 ± 80 | 76 ± 8 | 4,000 ± 1,000 | 0.75 ± 0.05 |
| +Mg/GLU | | | 100 ± 1 | 2,000 ± 100 | 2.00 ± 0.09 |
| Chlorprothixene | | | | | |
| −Mg/GLU | 34 ± 6 | 200 ± 97 | 63 ± 6 | 7,700 ± 1,600 | 0.70 ± 0.05 |
| +Mg/GLU | | | 101 ± 1 | 8,100 ± 500 | 1.54 ± 0.06 |
| Desipramine | | | | | |
| −Mg/GLU | | | 100 ± 3 | 2,000 ± 200 | 0.72 ± 0.04 |
| +Mg/GLU | | | 98 ± 1 | 8,000 ± 500 | 0.99 ± 0.04 |
| Dextromethorphan | | | | | |
| −Mg/GLU | 23 ± 3 | 34 ± 17 | 79 ± 3 | 3,000 ± 300 | 0.72 ± 0.04 |
| +Mg/GLU | | | 99 ± 1 | 2,000 ± 40 | 0.92 ± 0.01 |
| Dextrorphan | | | | | |
| −Mg/GLU | 79 ± 9 | 960 ± 200 | 20 ± 9 | 20,000 ± 15,000 | 0.77 ± 0.03 |
| +Mg/GLU | 100 ± 1 | 570 ± 200 | | | 0.94 ± 0.01 |
| DTG | | | | | |
| −Mg/GLU | 38 ± 10 | 560 ± 310 | 62 ± 10 | 12,000 ± 3,000 | 0.70 ± 0.05 |
| +Mg/GLU | | | 100 ± 1 | 15,000 ± 500 | 0.93 ± 0.02 |
| Imipramine | | | | | |
| −Mg/GLU | 30 ± 4 | 170 ± 60 | 68 ± 4 | 8,000 ± 1,000 | 0.63 ± 0.02 |
| +Mg/GLU | | | 100 ± 1 | 21,000 ± 1,000 | 1.3 ± 0.06 |
| Opipramol | | | | | |
| −Mg/GLU | 28 ± 2 | 110 ± 33 | 72 ± 2 | 25,000 ± 2,000 | 0.49 ± 0.02 |
| +Mg/GLU | | | 99 ± 1 | 158,000 ± 10,000 | 0.76 ± 0.06 |
| Promethazine | | | | | |
| −Mg/GLU | 23 ± 4 | 150 ± 70 | 75 ± 4 | 8,000 ± 1,000 | 0.75 ± 0.04 |
| +Mg/GLU | | | 101 ± 1 | 30,000 ± 2,000 | 1.8 ± 0.11 |
| Pyrilamine | | | | | |
| −Mg/GLU | 49 ± 4 | 850 ± 180 | 51 ± 4 | 49,000 ± 10,000 | 0.50 ± 0.02 |
| +Mg/GLU | | | 99 ± 1 | 336,000 ± 28,000 | —* |
| Spiperone | | | | | |
| −Mg/GLU | 51 ± 1 | 3000 ± 1400 | 46 ± 17 | 32,000 ± 15,000 | 0.78 ± 0.04 |
| +Mg/GLU | | | 104 ± 2 | 28,000 ± 3,000 | 2.5 ± 0.18 |
| Trifluoperazine | | | | | |
| −Mg/GLU | 37 ± 4 | 530 ± 170 | 61 ± 4 | 25,000 ± 4,000 | 0.58 ± 0.03 |
| +Mg/GLU | | | 104 ± 1 | 28,000 ± 2,000 | 3.1 ± 0.17 |
| Trihexyphenidyl | | | | | |
| −Mg/GLU | 23 ± 7 | 290 ± 190 | 76 ± 7 | 7,300 ± 1,300 | 0.79 ± 0.04 |
| +Mg/GLU | | | 101 ± 1 | 12,000 ± 500 | 1.21 ± 0.04 |
| Trimeprazine | | | | | |
| −Mg/GLU | 37 ± 4 | 230 ± 70 | 65 ± 4 | 12,000 ± 2,000 | 0.62 ± 0.03 |
| +Mg/GLU | | | 103 ± 1 | 42,000 ± 2,000 | 1.6 ± 0.09 |
| Tripelennamine | | | | | |
| −Mg/GLU | 53 ± 4 | 1000 ± 200 | 45 ± 4 | 53,000 ± 14,000 | 0.54 ± 0.03 |
| +Mg/GLU | | | 104 ± 1 | 28,000 ± 2,000 | 3.1 ± 0.1 |

* Indicates that a meaningless number was generated by computer analysis due to the low affinity of the compound.

tion curve for dextrorphan was shifted to the *left* when $Mg^{2+}$ and L-GLU were included in the binding assay, similar to that observed for PCP and MK-801. Computer analysis revealed that a two-component binding described the data significantly better than a one-component model in the absence of $Mg^{2+}$ and L-GLU but not in the presence of these compounds.

To further examine the specificity of compounds to interact with the multiple [$^3$H]TCP binding components, a series of other compounds with central nervous system activity were examined. Spiperone, propranolol, and the σ receptor-selective compound DTG (26) produced a binding profile similar to those of the tricyclic compounds described above (Table 1). Shallow inhibition curves were generated under basal conditions, whereas steep inhibition curves that were shifted to the right were obtained when $Mg^{2+}$ and L-GLU were included in the binding assay.

In contrast, a series of other compounds at a concentration of 10 μM, both in the absence and presence of $Mg^{2+}$ and L-GLU, did not inhibit specific binding. These included D-EKC, phenobarbital, metrazole, diazepam, verapamil, naloxone,

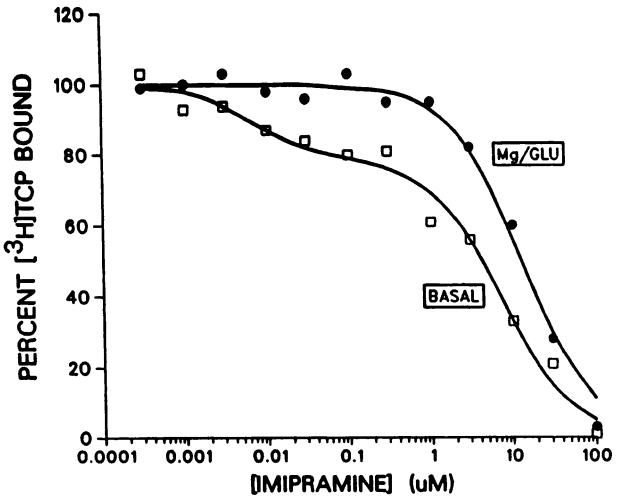


**Fig. 1.** Inhibition of [$^3$H]TCP binding by the TCA imipramine. Values shown are representative of three replicate experiments. Computer analysis indicated that a two-component binding model fit the data significantly better than a one-component model only under basal conditions.

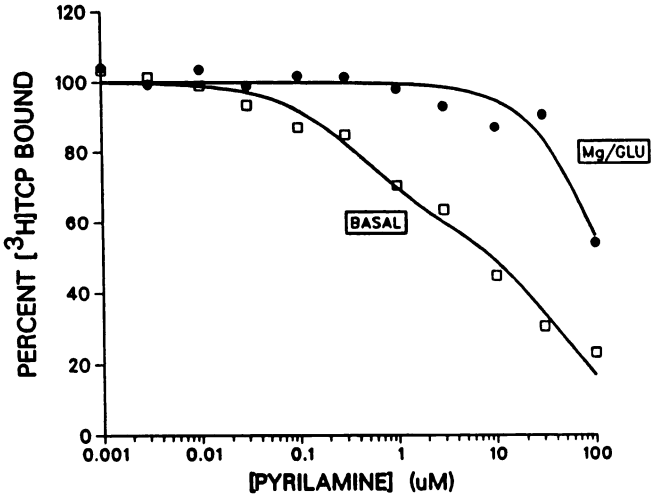


**Fig. 3.** Inhibition of [$^3$H]TCP binding by the antihistamine pyrilamine. Values shown are representative of three replicate experiments. Computer analysis indicated that a two-component binding model fit the data significantly better than a one-component model only under basal conditions.

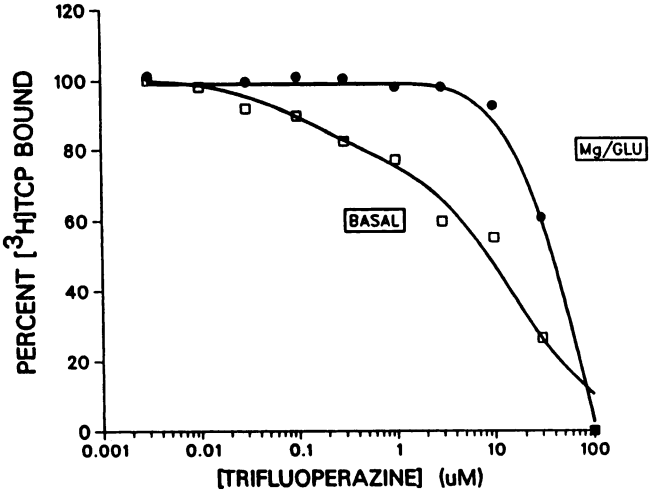


**Fig. 2.** Inhibition of [$^3$H]TCP binding by the phenothiazine antipsychotic trifluoperazine. Values shown are representative of three replicate experiments. Computer analysis indicated that a two-component binding model fit the data significantly better than a one-component model only under basal conditions.

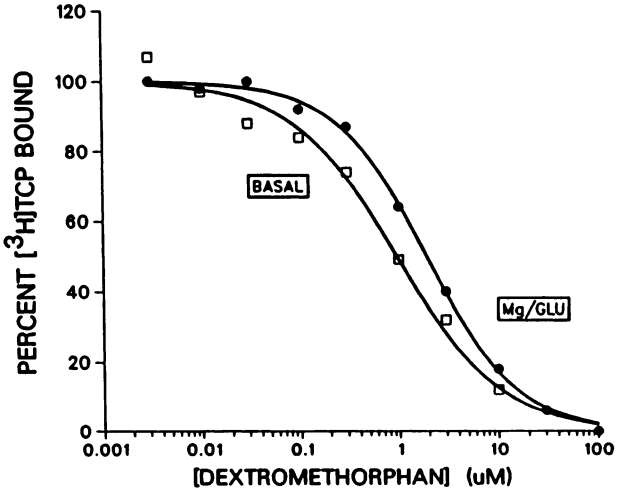


**Fig. 4.** Inhibition of [$^3$H]TCP binding by dextromethorphan. Values shown are representative of three replicate experiments. Computer analysis indicated that a two-component binding model fit the data significantly better than a one-component model only under basal conditions.

GABA, methysergide, sulpiride, dilantin sodium, atropine sulfate, and carbamazepine.

## Discussion

The present study demonstrates that compounds exist that inhibit [$^3$H]TCP binding more potently in the absence than in the presence of $Mg^{2+}$ and L-GLU. Interestingly, this is in contrast to what was previously found for dissociative anesthetics, such as dexoxadrol and PCP, and the novel anticonvulsant MK-801, which inhibited binding more potently in the presence of $Mg^{2+}$ and L-GLU (18). These findings suggest that compounds like the TCAs and dextromethorphan may be able to block conduction of ions through the NMDA-associated ion channel in a manner different from the dissociative anesthetics.

Previous work from our laboratory has revealed that multiple states of the PCP receptor exist (18). In that study, it was found that, using 5 nM [$^3$H]TCP in competition experiments, two binding components were detected in the absence of $Mg^{2+}$ and L-GLU, whereas essentially only one binding component was detected in the presence of $Mg^{2+}$ and L-GLU. Results from saturation experiments indicated that $Mg^{2+}$ and L-GLU converted a portion of low affinity binding into a high affinity state so that, at 5 nM [$^3$H]TCP, most if not all of the binding occurred to the high affinity state of the PCP receptor. The inhibition curves for the dissociative anesthetics and MK-801 were found to become steeper and shift to the left upon the addition of $Mg^{2+}$ and L-GLU. Because the high affinity state is essentially the only state of the PCP receptor present under these conditions, these results suggest that compounds such as PCP and MK-801 are selective for the high affinity state of the receptor.

Studies from a number of laboratories have indicated that PCP and MK-801 are open-channel blockers (16, 17), i.e., the NMDA ion channel must first be opened by an NMDA agonist, such as L-GLU, before these noncompetitive blockers can elicit

their effects. Because inclusion of an NMDA agonist will also induce the formation of the high affinity state of the PCP receptor, enhancing the affinity for compounds to interact at the PCP receptor, it is not unlikely that the opening of the channel is associated with the formation of the high affinity state of the PCP receptor.

In the present study, the inhibition curves for a number of compounds were found to shift *not* to the left but to the right when $Mg^{2+}$ and L-GLU were included in the binding assay. Again, because the high affinity state of the PCP receptor is essentially the only state present under these conditions, these results indicate that these compounds are selective for the *low* affinity state of the PCP receptor. The significance of pharmacological activity at the low affinity state of the PCP receptor is unclear. However, if the high affinity state of the PCP receptor is associated with the open NMDA ion channel, the low affinity state of the PCP receptor may be associated with the closed NMDA ion channel. As previously suggested for dextromethorphan (27), compounds that inhibit binding more potently in the absence of $Mg^{2+}$ and L-GLU may be "closed-channel blockers."

Multiple affinity states of receptors have been demonstrated in numerous receptor systems, including the $\beta$-adrenergic (28), serotoninergic (29), and dopaminergic (30) receptors. These systems involve agonist interactions with receptors coupled to regulatory G proteins. In such instances, where multiple states of a receptor occur, agonists show selectivity for the high affinity receptor state. Antagonists generally do not discriminate between the high and low affinity states of a receptor, so it is unusual for compounds to selectively interact with the low affinity state of a receptor (31).

Similar to the G protein system, agonists at the PCP receptor, such as PCP and dexoxadrol, appear to selectively interact with the high affinity state of the PCP receptor (18). However, several other compounds, including the novel anticonvulsant MK-801 (18), also appear to selectively interact with this receptor state. In the present study, dextrorphan, a metabolite of dextromethorphan, was found to selectively interact with the high affinity state of the PCP receptor. Although the dissociative anesthetics could be classified as agonists at the PCP receptor, it is uncertain whether MK-801 or dextrorphan fit into this category. However, both compounds have been found to block glutamate-induced neurotoxicity (24). Thus, compounds selective for the high affinity state of the PCP receptor appear to block NMDA-induced responses. Whether dextrorphan produces its effect through a competitive interaction at the PCP receptor, as do PCP and dexoxadrol, is presently unclear.

A number of compounds, as shown in Table 1, inhibit binding more potently in the absence of $Mg^{2+}$ and L-GLU and appear to be selective for the low affinity state of the PCP receptor. At present, the significance of the differences in the percentage of high and low affinity binding components between compounds is unclear. For example, the percentage of the low affinity state was 23 for dextromethorphan but 53 for tripelennamine. In G protein-coupled systems, such as the $\beta$-adrenergic receptor in frog erythrocytes, it has been suggested that the proportion of high affinity state formation is proportional to the intrinsic activity of the agonist (32). This could be a contributing factor in the present study. Another possibility is that a loss of binding to the low affinity state of the receptor could occur during filtration. However, this does not seem to be relevant because the $B_{max}$ values obtained in the absence (low affinity state only) and presence (high and low affinity states) of $Mg^{2+}$ and L-GLU were similar (18).

Results from Table 1 also reveal that the Hill coefficient values for a number of compounds, particularly in the presence of $Mg^{2+}$ and L-GLU, appeared to be greater than 1. This may be due to membrane-destabilizing effects of the compounds at the high concentrations needed to inhibit [$^3$H]TCP binding.

What is the significance then, of compounds being selective for the low affinity state of the PCP receptor? There is growing evidence that these compounds are involved in mediating NMDA-induced effects. A number of compounds, including the antitussive dextromethorphan and the antimuscarinic antiparkinsonian compound trihexyphenidyl, block glutamate-induced neurotoxicity in the mouse cortical neuron (24) and in the *ex vivo* chick embryo retina (25) assays, respectively. Interestingly, another antimuscarinic antiparkinsonian compound, benztropine, was also found to be selective for the low affinity state of the PCP receptor. In contrast, the antimuscarinic compound atropine was unable to inhibit [$^3$H]TCP binding (Table 2), indicating that the low affinity state of the receptor is not exclusively selective for antimuscarinic activity. Furthermore, Leander (33) has recently reported that TCAs, which Reynolds and Miller (23) previously suggested interact at the zinc site within the NMDA receptor complex, block NMDA-induced neurotoxicity in mice.

Dextromethorphan has also been found to protect against the effects of hypoxic ischemia (20), reduce kindled amygdala seizures (34), and inhibit NMDA-induced convulsions (35). This is particularly noteworthy because the NMDA receptor is thought to be involved in mediating convulsant and ischemic effects (36). The present findings extend the hypothesis that these compounds are acting at the NMDA receptor complex. It remains to be seen whether all compounds with selectivity for the low affinity state of the PCP receptor are neuroprotective.

Interestingly, most of the compounds selective for the low affinity state in the present study were shown to potently inhibit [$^3$H]dextromethorphan binding in guinea pig brain (37). This is especially intriguing because dextromethorphan was one of the most potent compounds ($IC_{50}$ = 34 nM) in inhibiting

TABLE 2

**Comparison of $IC_{50}$ values**

Spearmann-Rho = 0.60; $p < 0.05$.

| | [$^3$H]TCP Binding −Mg/L-GLU, $IC_{50_H}$ | [$^3$H]Dextromethorphan Binding, $IC_{50}$* |
|---|---|---|
| | nM | |
| Dextromethorphan | 34 | 25 |
| Opipramol | 110 | 7 |
| Benztropine | 120 | 36 |
| Chlorpromazine | 120 | 35 |
| Promethazine | 150 | 290 |
| Imipramine | 170 | 100 |
| Trimeprazine | 230 | 54 |
| Amitriptyline | 290 | 47 |
| Trihexyphenidyl | 290 | 1000 |
| Trifluoperazine | 530 | 12 |
| Pyrilamine | 850 | 300 |
| Tripelennamine | 1000 | 510 |
| Propranolol | 2800 | 145 |
| Spiperone | 3000 | 285 |

* Values from Craviso and Musacchio (37).

binding to the low affinity state of the PCP receptor. When the $IC_{50}$ values obtained for the two binding components under basal binding conditions and for the one binding component in the presence of $Mg^{2+}$ and L-GLU in the present study were compared with the $IC_{50}$ values obtained from the inhibition of [$^3$H]dextromethorphan binding (37), a significant correlation (Spearman-Rho, $r = 0.60$; $p < 0.05$) was found between the $IC_{50}$ values for [$^3$H]dextromethorphan binding and the $IC_{50}$ values for the high affinity component in the [$^3$H]TCP binding assay in the absence of $Mg^{2+}$ and L-GLU (Table 2). These data suggest that [$^3$H]dextromethorphan may be labeling the low affinity state of the PCP receptor. The finding that several compounds, such as trihexyphenidyl or trifluoperazine, differed more than other compounds in the two systems may be a function of their specific sites of action within the NMDA receptor complex, which is unclear at present.

In summary, compounds exist that inhibit the binding of [$^3$H]TCP more potently in the absence of $Mg^{2+}$ and L-GLU. Because open channel blockers such as PCP and MK-801 are more potent in the presence of these agents, these results may help to explain how certain compounds, such as dextromethorphan and trihexyphenidyl, block NMDA-induced effects and provide hope that compounds can be developed that do not possess PCP-like side effects.

## References


1. Simon, R. P., J. H. Swan, T. Griffiths, and B. S. Meldrum. Blockade of *N*-methyl-D-aspartate receptors may protect against ischemic damage in the brain. *Science (Wash. D. C.)* **226**:850–852 (1984).
2. Rothman, S. M., and J. W. Olney. Excitotoxicity and the NMDA receptor. *Trends Neurosci.* **10**:299–302 (1987).
3. Croucher, M., J. Collins, and B. Meldrum. Anticonvulsant action of excitatory amino acid antagonists. *Science (Wash. D. C.)* **216**:899–901 (1982).
4. Anis, N. A., S. C. Berry, N. R. Burton, and D. Lodge. The dissociative anesthetics, ketamine and phencyclidine, selectively reduce excitation of central mammalian neurones by *N*-methyl-D-aspartate. *Br. J. Pharmacol.* **79**:565–575 (1983).
5. Honey, C. R., Z. Miljkovic, and J. F. MacDonald. Ketamine and phencyclidine cause a voltage-dependent block of responses to L-aspartic acid. *Neurosci. Lett.* **61**:135–139 (1985).
6. Johnson, J. W., and P. Ascher. Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature (Lond.)* **325**:529–531 (1987).
7. Reynolds, I. J., S. N. Murphy, and R. J. Miller. $^3$H-labeled MK-801 binding to the excitatory amino acid receptor complex from rat brain is enhanced by glycine. *Proc. Natl. Acad. Sci. USA* **84**:7744–7748 (1987).
8. Nowak, L., P. Bregestovski, P. Ascher, A. Herbet, and A. Prochiantz. Magnesium gates glutamate-activated channels in mouse central neurones. *Nature (Lond.)* **307**:462–465 (1984).
9. Mayer, M. L., G. L. Westbrook, and P. B. Guthrie. Voltage-dependent block by $Mg^{2+}$ of NMDA responses in spinal cord neurones. *Nature (Lond.)* **309**:261–263 (1984).
10. Westbrook, G. L., and M. L. Mayer. Micromolar concentrations of $Zn^{2+}$ antagonize NMDA and GABA responses of hippocampal neurons. *Nature (Lond.)* **328**:640–643 (1987).
11. Peters, S., J. Koh, and D. W. Choi. Zinc selectively blocks the action of *N*-methyl-D-aspartate on cortical neurons. *Science (Wash. D. C.)* **236**:589–593 (1987).
12. MacDermott, A. B., M. L. Mayer, G. L. Westbrook, S. J. Smith, and J. L. Barker. NMDA-receptor activation increases cytoplasmic calcium concentration in cultured spinal cord neurones. *Nature (Lond.)* **321**:519–522 (1986).
13. Jahr, C. E., and C. F. Stevens. Glutamate activates multiple single channel conductances in hippocampal neurones. *Nature (Lond.)* **325**:522–525 (1987).
14. Clineschmidt, B. V., M. Williams, J. J. Witoslawski, P. R. Bunting, E. A. Risley, and J. A. Totaro. Restoration of shock suppressed behavior by treatment with (+)-4-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]-cycloheptene-5,10-imine (MK-801), a substance with potent anticonvulsant, central sympathomimetic and apparent anxiolytic properties. *Drug. Dev. Res.* **2**:147–151 (1982).
15. Kemp, J. A., A. C. Foster, and E. H. F. Wong. Non-competitive antagonists of excitatory amino acid receptors. *Trends Neurosci.* **10**:294–298 (1987).
16. Huettner, J. E., and B. P. Bean. Block of *N*-methyl-D-aspartate-activated current by the anticonvulsant MK-801: selective binding to open channels. *Proc. Natl. Acad. Sci. USA* **85**:1307–1311 (1988).
17. MacDonald, J. F., Z. Miljkovic, and P. Pennefather. Use-dependent block of excitatory amino acid currents in cultured neurons by ketamine. *J. Neurophysiol.* **58**:251–266 (1987).
18. Loo, P. S., A. F. Braunwalder, J. Lehmann, M. Williams, and M. A. Sills. Interaction of L-glutamate and magnesium with the phencyclidine sites in rat brain: evidence for multiple affinity states of the phencyclidine/*N*-methyl-D-aspartate receptor complex. *Mol. Pharmacol.* **32**:820–830 (1987).
19. Goldberg, M. P., P.-C. Pham, and D. W. Choi. Dextrorphan and dextromethorphan attenuate hypoxic injury in neuronal culture. *Neurosci. Lett.* **80**:11–15 (1987).
20. Prince, D. A., and H. R. Feeser. Dextromethorphan protects against cerebral infarction in a rat model of hypoxia-ischemia. *Neurosci. Lett.* **85**:291–296 (1988).
21. George, C. P., M. P. Goldberg, D. W. Choi, and G. K. Steinberg. Dextromethorphan reduces neocortical ischemic neuronal damage *in vivo*. *Brain Res.* **440**:375–379 (1988).
22. Choi, D. W., S. Peters, and V. Viseskul. Dextrorphan and levorphanol selectively block *N*-methyl-D-aspartate receptor-mediated neurotoxicity on cortical neurons. *J. Pharmacol. Exp. Ther.* **242**:713–720 (1987).
23. Reynolds, I. J., and R. J. Miller. Tricyclic antidepressants block *N*-methyl-D-aspartate receptors: similarities to the action of zinc. *Br. J. Pharmacol.* **95**:95–102 (1988).
24. Choi, D. W. Dextrorphan and dextromethorphan attenuate glutamate neurotoxicity. *Brain Res.* **403**:333–336 (1987).
25. Olney, J. W., M. T. Price, J. Labruyere, K. S. Salles, G. Frierdich, M. Mueller, and E. Silverman. Antiparkinsonian agents are phencyclidine agonists and *N*-methyl-aspartate antagonists. *Eur. J. Pharmacol.* **142**:319–320 (1987).
26. Weber, E., M. Sonders, M. Quarum, S. McLean, S. Pou, and J. F. W. Keana. 1,3-Di(2-[5-$^3$H]tolyl)guanidine: a selective ligand that labels *sigma*-type receptors for psychotomimetic opiates and antipsychotic drugs. *Proc. Natl. Acad. Sci. USA* **83**:8784–8788 (1986).
27. Lehmann, J., M. A. Sills, C. Tsai, P. S. Loo, and D. A. Bennett. Dextromethorphan modulates the NMDA-type receptor-associated ion channel by binding to its closed state, in *Frontiers in Excitatory Amino Acid Research* (E. A. Cavalheiro, J. Lehmann, and L. Turski, eds.). Alan R. Liss, New York, 571–578 (1988).
28. DeLean, A., J. M. Stadel, and R. J. Lefkowitz. A ternary complex model explains the agonist-specific binding properties of the adenylate cyclase-coupled $\beta$-adrenergic receptor. *J. Biol. Chem.* **255**:7108–7117 (1980).
29. Sills, M. A., B. B. Wolfe, and A. Frazer. Multiple states of the 5-hydroxytryptamine$_1$ receptor as indicated by the effects of GTP on [$^3$H]5-hydroxytryptamine binding in rat frontal cortex. *Mol. Pharmacol.* **26**:10–18 (1984).
30. DeLean, A., B. F. Kilpatrick, and M. G. Caron. Dopamine receptor of the porcine anterior pituitary gland: evidence for two affinity states discriminated by both agonists and antagonists. *Mol. Pharmacol.* **22**:290–297 (1982).
31. Abramson, S. N., and P. B. Molinoff. *In vitro* interactions of agonists and antagonists with *beta*-adrenergic receptors. *Biochem. Pharmacol.* **33**:869–875 (1984).
32. Kent, R. S., A. De Lean, and R. J. Lefkowitz. A quantitative analysis of *beta*-adrenergic receptor interactions: resolution of high and low affinity states of the receptor by computer modeling of ligand binding data. *Mol. Pharmacol.* **17**:14–23 (1980).
33. Leander, J. D. Tricyclic antidepressants block *N*-methyl-D-aspartic acid-induced lethality in mice. *Br. J. Pharmacol.* **96**:256–258 (1989).
34. Feeser, H. R., J. L. Kadis, and D. A. Prince. Dextromethorphan, a common antitussive, reduces kindled amygdala seizures in the rat. *Neurosci. Lett.* **86**:340–345 (1988).
35. Ferkany, J. W., S. A. Borosky, D. B. Clissold, and M. J. Pontecorvo. Dextromethorphan inhibits NMDA-induced convulsions. *Eur. J. Pharmacol.* **151**:151–154 (1988).
36. Goldberg, M. P., J. H. Weiss, P.-C. Pham, and D. W. Choi. *N*-Methyl-D-aspartate receptors mediate hypoxic neuronal injury in cortical culture. *J. Pharmacol. Exp. Ther.* **243**:784–791 (1987).
37. Craviso, G. L., and J. M. Musacchio. High affinity dextromethorphan binding sites in guinea pig brain. II. Competition experiments. *Mol. Pharmacol.* **23**:629–640 (1983).


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