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Synthesis and characterization of a series of diarylguanidines that are noncompetitive *N*-methyl-D-aspartate receptor antagonists with neuroprotective properties

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ABSTRACT **Four diarylguanidine derivatives were synthesized. These compounds were found to displace, at submicromolar concentrations, ^{3}H-labeled 1-[1-(2-thienyl)cyclohexyl]piperidine and (+)-[^{3}H]MK-801 from phencyclidine receptors in brain membrane preparations. In electrophysiological experiments the diarylguanidines blocked *N*-methyl-D-aspartate (NMDA)-activated ion channels. These diarylguanidines also protected rat hippocampal neurons *in vitro* from glutamate-induced cell death. Our results show that some diarylguanidines are noncompetitive antagonists of NMDA receptor-mediated responses and have the neuroprotective property that is commonly associated with blockers of the NMDA receptor-gated cation channel. Diarylguanidines are structurally unrelated to known blockers of NMDA channels and, therefore, represent a new compound series for the development of neuroprotective agents with therapeutic value in patients suffering from stroke, from brain or spinal cord trauma, from hypoglycemia, and possibly from brain ischemia due to heart attack.**

Phencyclidine (PCP) is an arylcyclohexylamine that was originally used as an anesthetic (1). For several years it has been known that there are specific binding sites for [^{3}H]PCP (PCP receptors) on mammalian brain neurons (2–4). Recently, studies in numerous laboratories have provided evidence that PCP receptors are associated with a nonselective cation channel that is gated by the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptor (4, 5). Drugs that bind to PCP receptors antagonize NMDA receptor-mediated responses noncompetitively by blocking the open ion channel of the receptor–channel complex (5). Known PCP receptor ligands include PCP analogs such as 1-[1-(2-thienyl)cyclohexyl]piperidine (TCP), benzomorphan (σ) opiates, dioxalones, benz[*f*]isoquinolines, and some bicyclic amines such as the drug MK-801 {(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine} (4, 6).

Most PCP receptor ligands also bind to σ receptors in mammalian brain membrane suspensions (4). A notable exception to this observation is MK-801. σ receptors do not appear to be associated with NMDA channels and their physiological function is still unknown. However, they are pharmacologically related to PCP receptors in that many PCP receptor ligands differentiate only poorly between σ and PCP receptors in brain membrane binding assays. Weber, Keana, and coworkers (7) have developed what appears to be the most selective σ receptor ligand identified to date. This compound, *N*,*N*′-di-(*o*-tolyl)guanidine (DTG), shows only negligible crossreactivity with PCP receptors (8). However, because of the extensive crossreactivity of many σ receptor ligands with PCP receptors (and vice versa), we reasoned that small chemical alterations to the DTG molecule might generate compounds with increased affinity for PCP receptors. We have now synthesized four analogs of DTG (Fig. 1): *N*,*N*′-di-(*m*-tolyl)guanidine (DMTG), *N*,*N*′-di-(*o*-ethylphenyl)guanidine (DOEPG), *N*,*N*′-di-(*m*-ethylphenyl)guanidine (DMEPG), and *N*,*N*′-di-(*o*-iodophenyl)guanidine (DOIPG). These compounds have submicromolar affinities for PCP receptors, block NMDA-receptor-coupled cation channels, and have neuroprotective properties in an *in vitro* neuroprotection model.

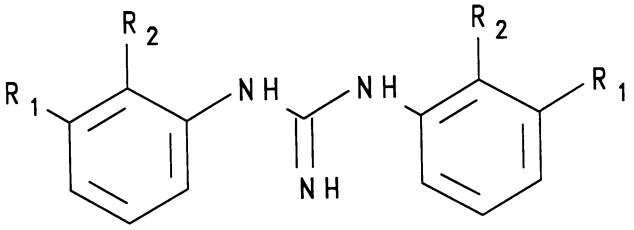

FIG. 1. The structural formulae of the N,N′-disubstituted guanidines tested in this study. For DTG, R_1 = H and R_2 = CH_3; for DMTG, R_1 = CH_3 and R_2 = H; for DOEPG, R_1 = H and R_2 = C_2H_5; for DMEPG, R_1 = C_2H_5 and R_2 = H; for DOIPG, R_1 = H and R_2 = I.

MATERIALS AND METHODS

Synthetic Chemistry. *Analytical methods and chemical supplies.* Melting points were determined in open capillary tubes on a Thomas–Hoover apparatus. Analyses for all compounds below were performed by Desert Analysis (Tucson, AZ). NMR spectra were recorded in $C^2H_3O^2H$ or C^2HCl_3 on a General Electric QE-300, and chemical shifts (δ) are reported in ppm relative to the residual signal of the deuterated solvent ($^1HC^2H_2O^2H$, 3.30 ppm). IR spectra were recorded on a Nicolet 5DXB FT-IR. All amines were purified by standard procedures or were used directly as received. CNBr (Aldrich) was used as obtained. Et_2O was routinely distilled from benzophenone ketyl.

Synthesis of DMTG. CNBr (788 mg, 7.44 mmol) was placed in a 25-ml round-bottom flask and *m*-toluidine (1.89 g, 17.6 mmol) was added dropwise. After the exothermic reaction

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Abbreviations: AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionate; CCP, 3-[1-(3-carboxy)piperazinyl]propane phosphonate; DMEPG, *N*,*N*′-(*m*-ethylphenyl)guanidine; DMTG, *N*,*N*′-di-(*m*-tolyl)guanidine; DOEPG, *N*,*N*′-di-(*o*-ethylphenyl)guanidine; DOIPG, *N*,*N*′-di-(*o*-iodophenyl)guanidine; DTG, *N*,*N*′-di-(*o*-tolyl)guanidine; NMDA, *N*-methyl-D-aspartate; PCP, phencyclidine; TCP, 1-[1-(2-thienyl)cyclohexyl]piperidine.

had subsided, the residue was taken up in CH_2Cl_2 (20 ml) and was extracted with 5% HCl (5 × 10 ml). The aqueous extracts were adjusted to pH 10 with 6 M NaOH. The resulting precipitate (674 mg, 38%) was filtered off and crystallized from EtOH/H_2O to give DMTG (240 mg, 14%) as white needles: melting point (mp) 105–106°C (literature mp 110°C, ref. 8). Anal. Calcd. for $C_{15}H_{17}N_3$: C, 75.28; H, 7.16; N, 17.56. Found: C, 75.42; H, 7.11; N, 17.43. ^{1}H NMR ($C^2H_3O^2H$): δ 2.289 (s, 6 H), 6.814 (d, 2 H, J = 7.5 Hz), 6.939 (d, 2 H, J = 7.5 Hz), 6.981 (s, 2 H), 7.141 (t, 2 H, J = 7.5 Hz). IR ($CHCl_3$) 3509, 3402, 1649, 1576 cm^{-1}.

Synthesis of DOEPG. A solution of CNBr (1.41 g, 13.3 mmol) in 95% EtOH (3 ml) was added to an ice-cold solution of 2-ethylaniline (3.08 g, 25.4 mmol) in 95% EtOH (10 ml). The reaction mixture was heated at 150°C under a rapid stream of N_2 for 30 min and was allowed to cool to 25°C. The resulting solid was dissolved in 95% EtOH (15 ml), and 10% NaOH (30 ml) was added. White needles formed and were filtered off and recrystallized twice from aqueous 25% EtOH, to give DOEPG as white needles (2.00 g, 59%): mp 158–161°C (literature mp 161.5–162°C, ref. 9). ^{1}H NMR (C^2HCl_3): δ 1.209 (t, 6 H, J = 7.5 Hz), 2.654 (q, 4 H, J = 7.5 Hz), 7.054–7.255 (m, 8 H). IR (C^2HCl_3): 2971, 1629, 1589, 1490, 1417, 1217 cm^{-1}.

Synthesis of DMEPG. A solution of CNBr (650 mg, 6.14 mmol) in Et_2O (1 ml) was added to 3-ethylaniline (1.42 g, 11.7 mmol). After the exothermic reaction had subsided, the viscous oil was heated under a stream of N_2 at 150°C for 15 min and then was allowed to cool to 25°C. The resulting solid was dissolved in 95% EtOH (20 ml), and 10% NaOH (20 ml) was added. A white precipitate formed and was filtered off and recrystallized twice from aqueous 50% EtOH, to give DMEPG as white needles (620 mg, 20%): mp 96–98°C. Anal. Calcd. for $C_{17}H_{21}N_3$: C, 76.37; H, 7.92; N, 15.72. Found: C, 75.93; H, 7.90; N, 15.76. ^{1}H NMR (C^2HCl_3): δ 1.216 (t, 6 H, J = 7.5 Hz), 2.608 (q, 4 H, J = 7.5 Hz), 6.937 (m, 6 H), 7.222 (t, 2 H, J = 7.8 Hz). IR (C^2HCl_3): 2971, 1629, 1589, 1490, 1417, 1217 cm^{-1}.

Synthesis of DOIPG. To a suspension of 2-iodoaniline (1.46 g, 6.67 mmol) in 95% EtOH (5 ml) was added a solution of CNBr (359 mg, 3.38 mmol) in 95% EtOH (2 ml). The resulting solution was stirred at reflux for 45 min and then was allowed to cool to 25°C overnight. The brown solution was diluted with 1 M NaOH (5 ml). A brown oil separated out, and the cloudy supernatant was decanted. On standing, the supernatant deposited 490 mg (32%) of DOIPG as short white needles: mp 151–158°C. The brown oil solidified on standing and was crystallized from EtOH/H_2O [1:1 (vol/vol), 10 ml], to give 540 mg (35%) of additional DOIPG: mp 140–148°C. Two recrystallizations from EtOH/H_2O gave the analytical sample as white needles: mp 161–162°C. Anal. Calcd. for $C_{13}H_{11}N_3I_2$: C, 33.72; H, 2.39; N, 9.07. Found: C, 33.80; H, 2.26; N, 8.78. ^{1}H NMR ($C^2H_3O^2H$): δ 6.790 (t, 2 H, J = 7.8 Hz), 7.304 (t, 2 H, J = 7.8 Hz), 7.506 (d, 2 H, J = 8.1 Hz), 7.817 (d, 2 H, J = 7.8 Hz). IR (KBr): 729, 753, 1456, 1502, 1572, 1613, 1647, 3056, 3387 cm^{-1}.

Radioligand Binding Assays. PCP receptor binding assays were performed as described (10) with (+)-[^{3}H]MK-801 [97 Ci/mmol, synthesized as described (10); 1 Ci = 37 GBq] or [^{3}H]TCP (55 Ci/mmol, New England Nuclear) as the radioligand, and with guinea pig (7) or rat brain (11) membranes as a source of receptors. In brief, for assays with rat brain membranes, thawed crude synaptic membranes were incubated at 1 mg of protein per ml with 0.01% Triton X-100 for 15 min at 32°C, then washed by centrifugation to reduce the endogenous amino acid concentrations; 1 μM glycine and L-glutamate were added to the binding assays to maximally stimulate the PCP receptor binding.

For (+)-[^{3}H]MK-801 binding, 1 nM radioligand was incubated with about 100 μg of membrane protein for 4 hr at room temperature. For [^{3}H]TCP binding, 2 nM radioligand was incubated with 800 μg of guinea pig or 200 μg of rat brain membrane protein for 45 min at room temperature. Nonspecific binding was defined as that remaining in the presence of 10 μM PCP or (+)-MK-801 for (+)-[^{3}H]MK-801 binding and in the presence of 10 μM PCP for [^{3}H]TCP binding. All assays were carried out in 5 mM Tris/HCl or Tris/acetate buffer (pH 7.4 at 25°C) and were stopped by rapid filtration through Whatman GF/B or Schleicher & Schuell no. 32 glass fiber filters (presoaked in 0.05% polyethylenimine).

σ receptor binding assays were performed with [^{3}H]DTG as radioligand and guinea pig brain membranes as the source of receptors as described (7). [^{3}H]CPP {3-[1-(3-carboxy)piperazinyl]propanephosphonate} binding to the NMDA-type glutamate receptor (12), [^{3}H]glycine binding to the strychnine-insensitive glycine site (13), high-affinity [^{3}H]kainate binding to the kainate-type glutamate receptor (14), and [^{3}H]AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionate) binding to the quisqualate-type glutamate receptor (11) were assayed using rat brain membranes prepared as described above (all radioligands were obtained from New England Nuclear). IC_{50} values were determined from displacement curves as described (7) or by nonlinear least-squares regression analysis.

Cell Culture. Dissociated rat hippocampal cultures were prepared by a modification of the method of Huettner and Baughman (15). The hippocampi were removed from 1- to 3-day-old rats (Sprague–Dawley) and placed in a Cl^--free dissociation medium based upon a formulation of Choi (16), supplemented with 1 mM kynurenic acid (Sigma) and 10 mM $MgSO_4$. The hippocampi were incubated twice for 20 min at 37°C in dissociation medium containing papain (Worthington) at 10 units/ml. The tissue then was incubated for three 5-min periods at 37°C with trypsin inhibitor (Sigma type II-O) at 10 mg/ml. The cells were dissociated by trituration in growth medium. For electrophysiological studies, cells were plated on 13-mm-diameter coverslips at a density of 10^4 cells per cm^2. For neuroprotection studies, 4.0–6.0 × 10^5 cells were plated as 0.15-ml droplets onto grids that had been stamped into the center of 35-mm dishes (Corning) by using a Mecanex BB-form (WPI, New Haven, CT) and then coated with poly(D-lysine) and laminin (Collaborative Research). Cells were left overnight to attach to the plate surface, then 1.5 ml of growth medium was added to each dish. The growth medium (modified from ref. 17) was Eagle's minimum essential medium (MEM, with Earle's salts) supplemented with 5% fetal bovine serum (CCL), 5% defined supplemented calf serum (HyClone), 50 mM glucose, 50 units of penicillin and 50 μg of streptomycin per ml, and Mito+ serum extender (Collaborative Research). The cells were maintained at 37°C in a humidified 5% CO_2 atmosphere. After 2–4 days in culture, non-neuronal cell division was arrested by 2–3 days of exposure to 5 μM 1-β-D-arabinofuranosylcytosine (Sigma). The cells were maintained in a medium that was similar to the growth medium but without the fetal bovine serum.

Electrophysiological Studies. All recordings were performed with whole-cell or outside-out patch-clamp configurations (18) on neurons prepared as described above and maintained in cell culture for 1–3 weeks. Fragments of the coverslips containing the cells were placed in a flow chamber (19) mounted on the stage of an inverted microscope (Zeiss IM35) and viewed with phase-contrast optics. The balanced salts solution (BSS) which superfused the cells contained, in mM, NaCl, 140; KCl, 3.5; $CaCl_2$, 1; Hepes, 10; glucose, 5; picrotoxinin, 0.02; and tetrodotoxin, 5 × 10^{-4} (pH adjusted to 7.4 with NaOH). Agonists and antagonists were dissolved in BSS and applied to neurons from a U-tube apparatus (20) supplied by a purpose-designed multiway tap (21).

In most experiments the patch pipette contained, in mM, $CsCH_3SO_3$, 120; CsCl, 10; EGTA, 10; and Hepes, 10 (pH

adjusted to 7.2 with CsOH). Experiments were performed at room temperature (20–25°C).

The membrane current output of an Axoclamp-1B (Axon Instruments, Burlingame, CA) was filtered at 4000 Hz (3-decibel, 8-pole Bessel filter, Frequency Devices, Haverhill, MA) and recorded on video tape by the pulse-code modulation technique (VR 100, Instrutech, Mineola, NY). The recorded currents were analyzed by hand or by using the Strathclyde Electrophysiology Software Package (Strathclyde University, Glasgow, U.K.)

***In Vitro* Neuroprotection Studies.** Sister cultures were used to ensure a similar cell density. Exposure to glutamate was carried out at 32–34°C in a Hepes-buffered control salts solution (CSS) similar to that reported (22) and buffered for pH 7.4 at 34°C. The cultures were washed twice with CSS and then incubated for 5 min in CSS containing 1 μM glycine (23) and the compound to be tested (the controls had 1 μM glycine only). CSS containing 1 μM glycine, the test drug, and a known concentration of glutamate (0–1000 μM) was then added by triple exchange and the cultures were incubated for 5 min. The cultures were washed four times with CSS and then twice with medium before being placed in the incubator overnight. The next day the cultures were washed twice with CSS and treated for 5 min with 0.4% trypan blue. The cultures were washed three times and the surviving cells were counted in the grid area by phase-contrast microscopy. For each glutamate concentration used (and the control), cell survival was expressed as a percentage of the highest cell count within the concentration range, and the results were plotted against the appropriate glutamate concentration. Cultures not exposed to glutamate generally had between 4500 and 5500 surviving cells in the grid area.

RESULTS

Radioligand Binding Studies. DTG, DMTG, DOEPG, DMEPG, and DOIPG were tested for binding to guinea pig brain and rat brain PCP receptors and guinea pig brain σ receptors by using selective ^{3}H-labeled ligands for each of the two receptors (see *Materials and Methods*). DMTG, DOEPG, DMEPG, and DOIPG had submicromolar affinities for PCP receptors as judged by their ability to displace the two selective PCP receptor ligands from brain membranes (Tables 1 and 2). The parent compound DTG, by comparison, had a low affinity for PCP receptors.

None of the diarylguanidines tested showed significant binding toward the NMDA-type, kainate-type, or quisqualate-type glutamate binding sites, assayed by using [^{3}H]CPP, [^{3}H]kainate, and [^{3}H]AMPA, respectively, as specific radioligands. The diarylguanidines tested did not cause >50% inhibition in these binding assays even at 100 μM. Further, we observed <50% inhibition of strychnine-insensitive [^{3}H]glycine (23) binding at concentrations of the diarylguanidines up to 100 μM. It appears therefore that the only property of these diarylguanidines that is consistent with their neuroprotective properties (see below) is their affinity for the PCP receptor.

Table 1. Displacement by diarylguanidines of PCP and σ receptor radioligands from guinea pig brain membranes

Compound	Diarylguanidine IC_{50} [mean ± SEM (*n*)], nM		
	[^{3}H]MK-801	[^{3}H]TCP	[^{3}H]DTG
DTG	6800 ± 860 (3)	6100 ± 700 (3)	31 ± 3 (4)
DMTG	280 ± 40 (3)	465 ± 30 (3)	43 ± 5 (3)
DOEPG	540 ± 76 (4)	455 ± 87 (4)	14 ± 1 (4)
DMEPG	189 ± 46 (3)	263 ± 70 (4)	8 ± 2 (5)
DOIPG	200 ± 40 (3)	260 ± 40 (3)	12 ± 1 (3)

Table 2. Displacement by diarylguanidines of PCP receptor radioligands from rat brain membranes

Compound	Diarylguanidine IC_{50} [mean ± SEM (*n*)], nM	
	[^{3}H]MK-801	[^{3}H]TCP
DTG	10,700 ± 2100 (3)	7800 ± 400 (4)
DMTG	330 ± 30 (4)	370 ± 30 (4)
DOEPG	745 ± 90 (4)	358 ± 53 (7)
DMEPG	168 ± 38 (6)	82 ± 10 (4)
DOIPG	240 ± 60 (4)	210 ± 60 (4)

All the diarylguanidines tested here retained a high affinity (nanomolar range) for σ receptors as judged by their ability to displace [^{3}H]DTG from guinea pig brain membrane binding sites (Table 1). This is in keeping with the observation that σ receptors tolerate a wide range of chemical alterations on compounds that are known to interact with these binding sites (24).

Electrophysiological Studies. All the diarylguanidines tested were able to depress the membrane current responses of cultured hippocampal neurons to NMDA (plus glycine). At 10 μM, the low-affinity PCP receptor ligand DTG caused a reduction of NMDA-induced responses that was similar to the inhibition that has been demonstrated with Mg^{2+} (25). On the other hand, the diarylguanidines with submicromolar affinities for the PCP receptor caused a use-dependent inhibition of NMDA-induced responses that was similar to the inhibition seen with MK-801 (26). For example, Fig. 2 shows the effect of DOIPG on NMDA-induced membrane current responses. Twenty-second applications of 20 μM NMDA in the presence of 1 μM glycine (control stimulus) resulted in an inward current at a holding potential of −60 mV, which faded only slightly during the application of the agonist (Fig. 2*A*). Repetitive applications (every 2 min) to the same cell produced currents that varied <10% in maximum amplitude over a period of at least 10 min. When 10 μM DOIPG was applied in conjunction with NMDA and glycine, the inward current initially achieved an amplitude approaching that of the control response but waned rapidly (Fig. 2*B*). Recovery from this inhibition required repeated applications of NMDA and glycine.

Our studies of the effects of the diarylguanidines on individual NMDA-receptor-coupled ion channels (data not shown) indicated that all the diarylguanidines tested can

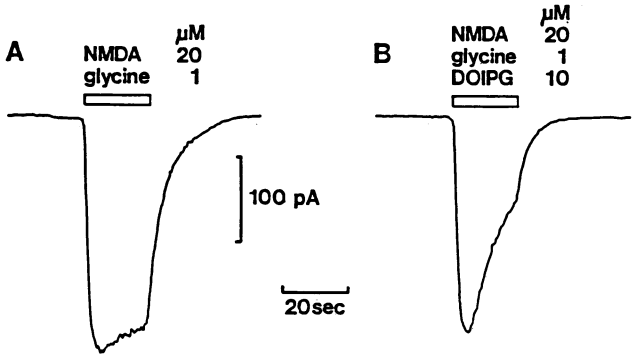

FIG. 2. Effect of 10 μM DOIPG on membrane currents induced by the application of 20 μM NMDA (plus 1 μM glycine) to a rat hippocampal neuron in cell culture. Both records were obtained at a holding potential of −60 mV and a temperature of 22°C. The records have been smoothed by a moving-average technique (Strathclyde Electrophysiology Software Package) to illustrate clearly the time course of the mean membrane current flow in response to the agonist. (*A*) Control response to NMDA (plus glycine). Note the slight fading of the response during the agonist presentation. (*B*) Response generated when DOIPG was included with NMDA (plus glycine). Note the rapid decline of the response during the agonist presentation. This behavior is due to the blockade of open channels by DOIPG.

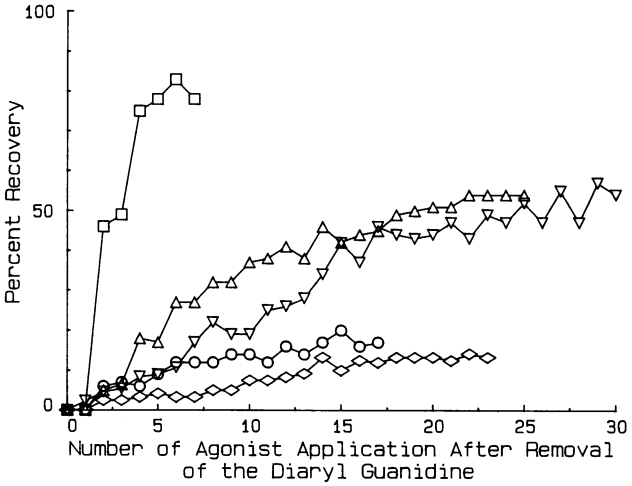

FIG. 3. Patterns of recovery of NMDA (20 μM) [plus glycine (1 μM)]-induced membrane current responses following their blockade by the diarylguanidines. The points plotted were derived from the equation $[(I_T - I_B)/(I_C - I_B)] \times 100$, where I_T is the peak amplitude of a test response to an agonist application after removal of the diarylguanidine from both bathing medium and agonist application, I_B is the peak amplitude of the response at the end of the period of exposure to the diarylguanidine, and I_C is the peak amplitude of the control responses to NMDA (plus glycine) before application of the diarylguanidine. Holding potential, −60 mV; temperature, 21–23°C. □, DTG; △, DMTG; ▽, DOIPG; ○, DOEPG; ◇, DMEPG.

block open channels. The difference between low-affinity and high-affinity ligands appears to be in the duration of the channel block. Low-affinity ligands cause a "flickery" block of an open channel, due to their short-lived interaction with the channel site (25). High-affinity ligands simply shorten the period of current flow following the opening of the channel, because once they have bound to the channel site they remain there for a period of time beyond the normal open time of the channel and, presumably, become trapped when the channel closes.

The use-dependent block of NMDA (plus glycine)-induced responses and use-dependent recovery seen with the high-affinity diarylguanidines was similar to that reported by Huettner and Bean (26) for the inhibition by MK-801 of NMDA (plus glycine)-induced responses in rat cortical neurons in cell culture. They concluded that the forward rate constant for open-channel block by MK-801 is largely governed by diffusion and that the high affinity of MK-801 for the PCP receptor results from a combination of a rapid association of MK-801 with the receptor followed by an extremely slow dissociation from the receptor. If this is true for all blockers of the NMDA-receptor ion channel, then the potencies of PCP receptor ligands (measured in radioligand binding studies) should be reflected in differences in the rate at which the channels are unblocked after removal of the channel-blocking agent. In preliminary studies examining the recovery of NMDA (plus glycine)-induced responses from inhibition by diarylguanidines, we found that the recovery from blockade was use-dependent and that many more agonist presentations were required to relieve the inhibition caused by the high-affinity ligands (DMTG, DOEPG, DMEPG, and DOIPG) than those necessary to restore responses inhibited by the low-affinity ligand, DTG (Fig. 3). This general finding is consistent with the notion that all the diarylguanidines are open-channel blockers of the NMDA receptor–channel complex and that their binding affinity for the PCP receptor site is reflected primarily in the rate at which they dissociate from the channel.

Neuroprotection Studies. DTG, DMTG, DOEPG, DMEPG, and DOIPG were tested for their neuroprotective properties *in vitro* against a range of glutamate concentrations (Fig. 4). Cultures tested with DMTG, DOEPG, DMEPG, and DOIPG (all at 30 μM) exhibited enhanced cell survival at glutamate concentrations of 30–1000 μM when compared to control cultures. The degree of neuroprotection afforded by three of these compounds (DMTG, DMEPG, and DOIPG), at the doses tested, was significant at the $P < 0.05$ level for glutamate concentrations of 30 μM or greater (analysis of variance and two-tailed t test). DOEPG was significantly neuroprotective only at 30 μM and 100 μM glutamate. By contrast, DTG (30 μM) failed to produce any significant neuroprotective response. These observations on the neuroprotective properties of the diarylguanidines tested in our experiments are consistent with their affinity for the PCP binding site in the brain and the inhibition of the NMDA current described above.

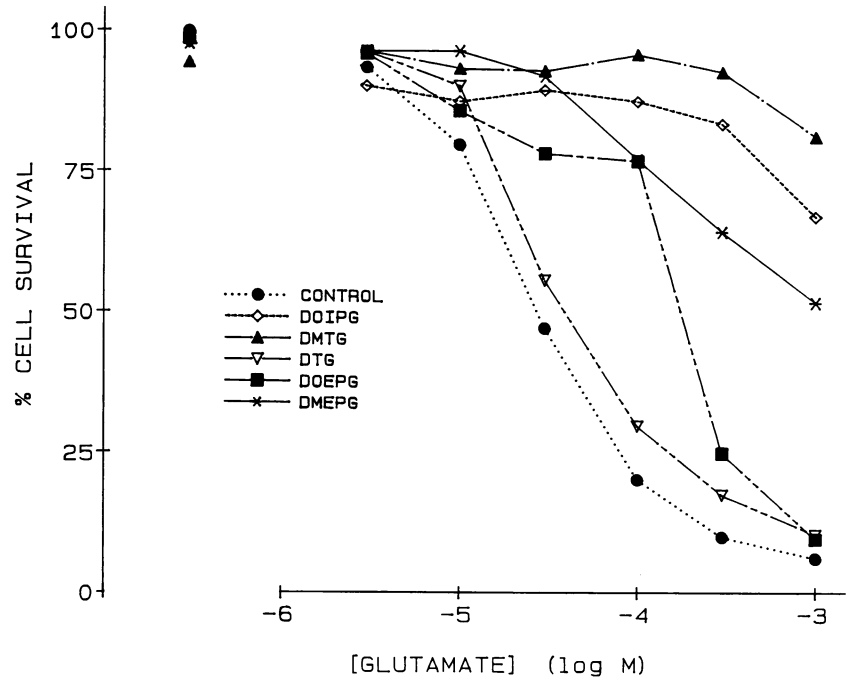

FIG. 4. Effect of the diarylguanidines (all at 30 μM) on the percentage of cell survival measured on the day following exposure of cell cultures from rat hippocampi to various glutamate concentrations. The cell counts for each series have been expressed as a percentage of the highest cell count in the series. Points in the control are the means of up to 13 separate experiments, whereas points in the test curves are the means of 4–6 separate experiments.

DISCUSSION

In this study we have described the synthesis of four blockers of NMDA channels that act by binding to PCP receptors. The PCP receptor is presumably located inside the ion channel of the NMDA receptor–channel complex, and ligands of the PCP receptor are thought to be open-channel blockers (27). That DMTG, DOEPG, DMEPG, and DOIPG bind to PCP receptors is evidenced by the ability of the ligands potently to displace [^{3}H]TCP and [^{3}H]MK-801 from PCP receptors in brain membranes.

As expected from the binding experiments, the diarylguanidines with high affinities ($IC_{50} < 1$ μM) for the PCP receptor blocked NMDA channels. The blockade of the channels by the high-affinity diarylguanidines was use-dependent; i.e., maintained or repeated applications of NMDA caused an increasing blockade of channels. This has been observed for other high-affinity NMDA channel blockers acting through PCP receptors, such as MK-801 (26), and is in keeping with the idea that these diarylguanidines block only open channels. The action of DTG as a "Mg^{2+}-like" inhibitor of NMDA-induced responses is entirely consistent with its low affinity for PCP receptors and probably unrelated to its high affinity for σ receptors. Although the function of σ receptors remains obscure, there is no evidence that they are linked in any way to NMDA receptor-coupled ion channels (ref. 4 and Table 1). However, that a number of diarylguanidines are also potent PCP receptor ligands is further evidence that PCP and σ receptors share some structural similarities.

NMDA channel blockers are known to have powerful neuroprotective properties in ischemic and hypoxic–ischemic brain injury models (28). Furthermore, NMDA channel blockers protect cultured neurons from glutamate-induced cell death (16, 22). Cell death in these models is believed to be caused by glutamate activation of NMDA receptors that results in excessive influx of Ca^{2+} into the cells. This leads to cell damage by the activation of intracellular cytotoxic processes (28). Thus, NMDA channel blockers have great potential as neuroprotective agents in stroke and heart attack/ischemia victims and in patients who have experienced brain or spinal cord trauma or periods of hypoglycemia (29). Since it has been suggested that the NMDA receptor may play a role in chronic neurodegenerative disorders, it is possible that NMDA receptor antagonists may also be useful in treating Huntington disease (30), amyotrophic lateral sclerosis (31), and Alzheimer disease (32). Because these compounds appear to block only those NMDA channels that are in the open state, they may be particularly effective in diseases that involve excessive excitation of these receptor-linked channels. As expected from the binding and electrophysiological studies, the high-affinity diarylguanidines did indeed have potent neuroprotective properties in cultured neurons exposed to exogenous glutamate.

The neuroprotective diarylguanidines reported here are chemically unrelated to any known NMDA channel blockers acting through PCP receptors. Previously, only compounds belonging to the PCP/ketamine series, benzomorphan opiates, dioxolanes, benzo[*f*]isoquinolines, and some bicyclic amines (including MK-801) were known to interact with PCP receptors. Some diarylguanidines must now be added to this list. We believe that substituted guanidines represent a new class of NMDA channel blockers, since we have observed that a number of other substituted guanidines also act as potent NMDA channel blockers (unpublished observations). Thus, disubstituted guanidines are important "lead" compounds for the development of NMDA receptor-coupled ion-channel blockers with neuroprotective properties.

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