Effects of Strychnine-Insensitive Glycine Receptor Ligands in Rats Discriminating Dizocilpine or Phencyclidine from Saline¹

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ABSTRACT

Several pharmacologically distinct sites are known to modulate the N-methyl-D-aspartate (NMDA) receptor/ion complex, including a site within the ion channel which binds uncompetitive antagonists like phencyclidine (PCP) or dizocilpine. Glycine acts as a co-agonist for activation of the NMDA receptor complex through a strychnine-insensitive receptor, which is a potential target for novel therapeutic agents (e.g., anticonvulsants, antidepressants). We evaluated the behavioral effects of glycine receptor ligands in rats trained to discriminate either dizocilpine or PCP from saline, to predict whether glycine receptor ligands might induce undesirable PCP-like subjective effects in humans. Dizocilpine (⁰MK-801), (⁻MK-801 and PCP produced dose-dependent substitution in these rats with potencies in accord with NMDA receptor affinity. Pentobarbital and drugs acting at other sites of the NMDA receptor, including competitive antagonists (NPC 12626 and LY 274614) and the polyamine antagonist, ifenprodil, did not substitute for either dizocilpine or PCP. In contrast to the uncompetitive antagonists like PCP, none of the strychnine-insensitive glycine receptor ligands substituted. Neither the full agonist, glycine; the partial agonists, 1-amino-1-cyclopropanecarboxylic acid, d-cycloserine or (⁺)-3-amino-1-hydroxypropylid-2-one; nor the antagonists, 7-chloro and 5,7-dichlorokynurenic acid, mimicked the discriminative stimulus effects of dizocilpine or PCP. Further, co-administration of 1-amino-1-cyclopropanecarboxylic acid did not significantly enhance the discriminative stimulus effects of dizocilpine. Intracerebroventricular administration of d-serine, a selective agonist of the strychnine-insensitive glycine receptor, neither mimicked nor blocked the discriminative stimulus effects of PCP. These data suggest that functional antagonists of the strychnine-insensitive glycine receptor may be devoid of the subjective side effects characteristic of NMDA channel ligands.

NMDA receptors have been implicated in the control of several important neurological functions. As such, NMDA antagonist has been considered as a strategy in the development of drugs for the treatment of anxiety, depression, epilepsy, stroke, cognitive deficits and drug dependence (see Olney, 1989; Wachtel and Turski, 1990; Toru et al., 1994; Witkin, 1995 for overview and references). However, many NMDA antagonists display side effects, in humans and non-human subjects, like those produced by the psychotomimetic, uncompetitive NMDA antagonist, PCP (cf. Balster and Willetts, 1988; Muir and Lees, 1995; Willetts et al., 1990; Witkin, 1995).

Agonist binding to the glycine site is critical to the gating of ions through the NMDA receptor-associated ion channel (Johnson and Ascher, 1987; Kleckner and Dingledine, 1988), a cooperative interaction that functions through allosteric coupling (cf. Johnson and Ascher, 1987; Kemp and Leeson, 1993; Kloog et al., 1990; Leeson and Iversen, 1994). Studies thus far have indicated that, in contrast to other NMDA antagonists, glycine-site antagonists do not generally produce the same profile of behavioral effects as caused by PCP. The functional glycine antagonists, ACPC, 7-CKA and (⁺)-HA-966 did not generally produce sedation, ataxia or locomotor stimulation that are induced by administration of PCP (see Carter, 1992; Witkin, 1995 for summaries). Compounds interacting with the strychnine-insensitive glycine receptor also did not impair learning or memory as reported with PCP and related compounds (Faiman et al., 1994). The preclinical efficacy of glycine site ligands at doses that are generally devoid of gross behavioral or neurological impairment has made the glycine site a focus of drug-discovery efforts (cf.

ABBREVIATIONS: ACPC, 1-amino-1-cyclopropanecarboxylic acid; CKA, chlorokynurenic acid; HA-966, 3-amino-1-hydroxypropylid-2-one; i.c.v., intracerebroventricular; LY 274614, (⁺)-(phosphonomethyl)-decahydroisoquinoline-3-carboxylic acid; MK-801, (⁺)-5-methyl-10,11-di-hydro-5H-dibenzo-a-d-cyclohepten-5,10-imine (dizocilpine); NPC 12626, (⁺)-2-amino-4,5-(1,2-cyclohexyl)-7-phosphonoheptanoic acid; PCP, phencyclidine.
Carter, 1992; Kemp and Leeson, 1993; Leeson and Iversen, 1994; Witkin, 1995). However, the possibility that glycine ligands will reproduce the subjective effects of PCP is unknown.

The first series of experiments in the present study was a systematic evaluation of the discriminative stimulus effects of structurally diverse glycine ligands (agonists, partial agonists and antagonists) to predict the possibility of PCP- or dizocilpine-like subjective effects (cf. Balster and Willetts, 1988; Holtzman, 1990; Willetts et al., 1990). Discriminations of both PCP and dizocilpine were studied because, whereas both compounds bind within the NMDA receptor ion channel, only PCP has significant interactions with the dopamine transporter (Reid et al., 1990; Harris, 1995). The data collected so far on this question are not consistent. In rats, neither (+)-HA-966 (Singh et al., 1990b; Witkin et al., 1995) nor 5,7-diCKA (Corbett and Dunn, 1993) substituted for the discriminative stimulus effects of PCP or dizocilpine at doses that are active in models predictive of therapeutic efficacy (e.g., as anticonvulsants). However, other data have suggested some overlap of the discriminative stimulus effects of PCP and glycine-site antagonists. Partial substitution for the discriminative stimulus effects of PCP was observed with ACPC and (+)-HA-966 in pigeons (Koek and Colpaert, 1992; Baron and Woods, 1995). The present experiment was, therefore, designed to compare systematically the discriminative stimulus effects of a range of glycine ligands over a broad range of biologically active doses.

Agonists of the strychnine-insensitive glycine site allosterically modulate the opening of the ion channel (Kloog et al., 1988). These compounds may thereby increase the probability of neural membrane depolarization and enhance the dissociation of voltage-dependent blockers like PCP (Ascher and Nowak, 1987; Salt, 1989; Thomson et al., 1989). The role of the glycine site in modulating the behavioral effects of PCP-like drugs was suggested by the blockade of PCP- or dizocilpine-induced motor effects by glycine (Toth and Lajtha, 1986; Evoniuk et al., 1991). Studies with selective glycine-site agonists have confirmed and extended these findings. The selective glycine-site agonists, d-serine and d-alanine (Johnson and Ascher, 1987; Johnson and Jones, 1990), have also been shown to block the stereotypy, ataxia and locomotor stimulation produced by PCP and dizocilpine (Contreras, 1990; Tannii et al., 1991, 1994). Because blockade of behavioral effects of PCP or dizocilpine has been used as a screen in the development of novel treatment strategies for psychoses (cf. Carter, 1992; Corbett, 1995; Potkin et al., 1992; Wachtel and Turski, 1990), further exploration of these compounds may provide useful lead candidates. Further, blockers of the behavioral effects of PCP have not been identified definitively. Therefore, in this second series of experiments, we examined the potential of d-serine to block the discriminative stimulus effects of PCP.

Methods

Animals. Male Sprague-Dawley rats (Charles River, Wilmington, MA) maintained at 350 g by postsession feeding were used. Water was continuously available for all rats in their living cages. All animals were housed in a temperature-controlled vivarium. All experiments were conducted during the light phase of a 12-hr light/dark cycle.

The facilities in which the animals were maintained are fully accredited by the American Association for the Accreditation of Labortatory Animal Care (AAALAC), and the studies described were conducted in accordance with the Guide for Care and Use of Laboratory Animals provided by the National Institutes of Health and adopted by the National Institute on Drug Abuse.

Apparatus. Experiments were conducted in operant-conditioning test chambers (BRS/LVE, model RTC-022). Two response levers, spaced 17 cm apart, were centered on the front panel to either side of a food pellet trough. A bank of three white lamps above the right lever and red lamps above the left lever could be illuminated. A white lamp at the top center of the front panel provided general illumination of the chamber. Chambers were enclosed within sound- and light-attenuating enclosures and supplied with white noise to mask extraneous sounds. Depression of the lever with a force exceeding 35 g (0.35 N) was recorded as a response and produced the click of a relay when the houselight and the stimulus lamps over the levers were illuminated.

Surgery. The rats to be trained to discriminate PCP were prepared with i.c.v. cannulae for use in the i.c.v. delivery of d-serine. Rats were anesthetized with Equithesin (3 ml/kg i.p.), placed in a Kopf stereotaxic frame and implanted with a guide cannula (22-gauge stainless steel, Plastic Products, Roanoke, VA) aimed at the right lateral ventricle. The cannula tip was positioned 2 mm above the ventricular lumen; 1.5 mm below the skull, −0.8 mm from Bregma, and 1.5 mm lateral to midline suture (Paxinos and Watson, 1982). The cannula was anchored to the skull with stainless steel screws and dental acrylic. Rats were permitted 1 week to recover from surgery before initiation of behavioral testing. Injections were made 2 mm below the implanted guide tip. Cannula placement was verified during surgery by observing the gravity flow of saline down PE-10 tubing connected to an injection cannula placed within the guide.

Drug discriminations. Rats were trained to discriminate intraperitoneal injections of either 0.2 mg/kg MK-801 from saline or 1.5 mg/kg PCP from saline. Six rats were used in each group. After drug administration, responses on only one lever produced food; after saline administration, responses on the opposite lever produced food. A 5-min (PCP experiments) or 10-min (MK-801 experiments) timeout occurred at the beginning of the session; during timeout the chamber was dark and responding had no scheduled consequences. After the timeout period, 20 consecutive responses on the injection-appropriate lever were required for food presentation. Responses on the alternate lever reset the response requirement. Once responding was stable, test sessions in which 20 consecutive responses on either lever produced food were conducted. Injections of test compounds were given no more than twice weekly. Because of expense and/or limited quantities of compounds, test compounds were not always given to both groups of rats.

Drugs. PCP HCl (National Institute on Drug Abuse, Rockville, MD), pentobarbital Na (Abbott Laboratories, Abbott Park, IL), 7-CKA (Research Biochemicals International, Natick, MA), 5,7-diCKA (Marion Merrell Dow Research Institute, Cincinnati, OH), dizocilpine [(-)-MK-801 maleate; Research Biochemicals International], (-)-MK-801 (Research Biochemicals International), d-cycloserine (Sigma Chemical Co., St. Louis, MO), glycine (Sigma), ifenprodil (Synthelabo Recherche, Bagneaux, France), LY 274614 (Research Biochemicals International) as part of the Chemical Synthesis Program of the National Institute of Mental Health, Contract 278-90-0007 (BS) and N01 MH30003, ACPC (Fluka Chemical Corp., Ronkonkoma, NY) and d-serine (Aldrich Chemical Co., Milwaukee, WI) were dissolved in distilled water. Solutions of ifenprodil were prepared with mild acidification. 7-CKA and 5,7-diCKA were prepared with aqueous base (pH = 10). For peripherally administered drugs, test compounds were injected (1 ml/kg i.p.), except d-cycloserine which was given subcutaneously. All systemically admin-
istered test compounds were given immediately before experimental sessions with the exception of several compounds noted below. Ifenprodil, NPC 12626 and LY 274614, were given 30 min before testing. ACPC was studied at various pretreatment times in the dizocilpine-trained rats. D-Serine was given 2 min before either saline or PCP. Injectionsof D-serine were made into the lateral ventricle (i.c.v.) at a volume of 5 μl over 2 min.

**Data analysis.** Experimental events and data collection were controlled by a PDP 11/73 computer operating under SKED behavioral software (State Systems, Inc., Kalamazoo, MI). Dose-effect functions were analyzed with data from the linear portion of the curves by analysis of variance in which linear regression provided ED₅₀ values and associated 95% confidence limits (Finney, 1964; Snedecor and Cochran, 1967). Comparisons of effects of each dose of drug to saline control values were made, with two-tailed Dunnett’s tests, after significant overall analyses of variance. *α* rates greater than .05 were considered to be nonsignificant.

**Results**

After training, behavior was under discriminative control of dizocilpine or PCP (~90% accuracy). The percentage of drug-lever responses under control conditions are shown in the top portions of tables 1 and 2. Response rates were lower in the presence of the training dose of dizocilpine (0.2 mg/kg) relative to saline (mean ± S.E.M. = 0.8 ± 0.3 vs. 1.9 ± 0.6 responses/sec).

Dizocilpine, (−)-MK-801 and PCP all produced dose-related increases in responding on the drug-associated lever in either dizocilpine (fig. 1, left top panel)- or PCP (fig. 1, right top panel)-discriminating rats. Higher doses of the training drugs decreased the percentage of drug-associated responses from that observed with the training dose. The reason for this latter result is not clear but may be related to the effects of this class of drugs on discrimination performance (cf. Koek et al., 1993; Witkin, 1995). The ED₅₀ value for dizocilpine with associated 95% confidence limits (in mg/kg) was 0.15 (0.13–0.17) and 0.15 (0.14–0.16) in the dizocilpine and PCP discriminations, respectively. For (−)-MK-801, ED₅₀ values were 0.56 (0.47–0.66) and 0.41 (0.29–0.59) for dizocilpine and PCP discriminations, respectively. For PCP, the ED₅₀ values were 1.50 (1.11–2.05) and 1.00 (0.84–3.30) for dizocilpine and PCP discriminations, respectively. All compounds also produced dose-dependent decreases in rates of responding (fig. 1, lower panels) except for PCP in PCP-trained rats.

None of the compounds that bind to the strychnine-insensitive glycine site produced significant drug-appropriate responding in either dizocilpine (table 1)- or PCP (table 2)-trained rats. Neither agonists (glycine), partial agonists (ACPC, D-cycloserine, (−)-HA-966) nor antagonists (7-CKA, 5,7-di-CKA) were effective in inducing either dizocilpine- or PCP-appropriate responses. Similar negative findings were obtained in time-course studies with the partial agonist ACPC. In these experiments, either 300 or 1000 mg/kg ACPC was administered at either 15 (table 1), 60 or 120 min before testing in dizocilpine-discriminating rats. The percentage of dizocilpine-appropriate responses in these tests was never greater than 25% (1000 mg/kg given 120 min before; data not shown). Despite the lack of substitution of the glycine-site ligands for dizocilpine or PCP, these drugs generally demonstrated effects on rates of responding or trends in that direction with higher doses.

Antagonists acting at the glutamate binding site (NPC 12626 or LY 274614) did not substitute for either dizocilpine or PCP (table 3). The sedative-hypnotic pentobarbital also did not substitute. Ifenprodil, an antagonist of the NMDA-associated polyamine site, also did not reproduce the discriminative stimulus effects of either dizocilpine or PCP.

Intracerebroventricular administration of D-serine (0.5–2 μmol) neither substituted for (fig. 2, open circles, left panel) nor blocked (fig. 2, filled circles, left panel) the discriminative stimulus effects of PCP. Response rates were not significantly affected by D-serine treatments (fig. 2, left, bottom panel).
studies, compounds with varied pharmacological actions at the glycine site (i.e., agonists, partial agonists, antagonists) did not engender PCP- or dizocilpine-like discriminative stimulus effects. These results suggest that therapeutically active doses of glycine-site compounds may not be associated with the motoric and subjective side-effect profile that has emerged in evaluation of functional antagonists of other sites on the NMDA receptor complex. Additional experiments indicated that these compounds may also not significantly modify the discriminative stimulus effects of PCP or dizocilpine when given in combination. The latter results suggest that the glycine site may not be a valid target for the discovery of antagonists of the subjective effects of PCP-like drugs.

Uncompetitive NMDA antagonists substituted for PCP or dizocilpine with a rank order of potency in accord with their affinities for the NMDA receptor ion channel (Wong et al., 1991), a finding consistent with previous reports (cf. Balster and Willetts, 1988; Tricklebank et al., 1987; Koek et al., 1990; France et al., 1991). Likewise, the lack of substitution of competitive NMDA antagonists, pentobarbital and the polyamine ligand, ifenprodil, is also generally consistent with previous findings (Jackson and Sanger, 1988; Sanger and Zivkovic, 1989; Koek et al., 1990; Willetts et al., 1990). Although the discriminative stimulus effects of LY 274614 can be differentiated from that of NPC 12626 (Willetts et al., 1993), neither of these competitive antagonists substituted for dizocilpine or PCP in the present study. Taken as a whole, these pharmacological data attest to the control of discriminative behavior by PCP and dizocilpine via the blockade of the NMDA-associated ion channel. The comparable results observed in both PCP- and dizocilpine-trained rats further underscores the common discriminative stimulus effects produced by these two compounds.

Other data (see the introduction) have suggested that glycine ligands do not produce the gross behavioral effects of PCP-like antagonists; results of the present study demonstrate that glycine receptor ligands also do not share the discriminative stimulus effects of PCP. These current findings are consistent with previous observations that neither (+)-HA-966 nor quinoxaline diamide glycine antagonists substituted in rats trained to discriminate PCP from saline (Singh et al., 1990b; Balster et al., 1995). Similar findings were also reported in mice discriminating dizocilpine from saline in which (+)-HA-966 did not substitute (Withkin et al., 1995). Nonetheless, data showing occasional or partial PCP-like discriminative stimulus effects of some glycine ligands, including (+)-HA-966 (Baron and Woods, 1995; Koek and Colpaert, 1992) have been reported when pigeons are used as research subjects. Further confirmation in mammalian species that glycine receptor ligands do not share discriminative stimulus effects with PCP-like drugs came from drug interaction experiments in the present experiment. In these experiments, high doses of ACPC did not augment the discriminative stimulus effects of dizocilpine. Together, these data suggest that compounds interacting with the NMDA receptor-associated glycine site may be devoid of the subjective effect profile that characterizes PCP and related dissociative anesthetics.

Given the relative difficulty with which some of the currently available glycine receptor ligands cross the blood-brain barrier, pharmacokinetic rather than pharmacody-
namic factors could account for the lack of PCP or dizocilpine-like discriminative stimulus effects (cf. Carter, 1992). However, several lines of evidence can be marshalled against the pharmacokinetic argument. Many of the compounds had effects on the rates of responding of the rats in the PCP or dizocilpine discriminations that may have been based upon their effects in the central nervous system. The glycine receptor ligands studied here have been shown to have central nervous system activity in rodents under other conditions within the range of doses studied here. Rats and mice can be trained to discriminate (+)-HA-966 (Singh et al., 1990b; Witkin et al., 1995). Under those conditions, two structurally unrelated glycine partial agonists, ACPC and D-cycloserine, fully substituted for the discriminative stimulus effects of (+)-HA-966 (Witkin et al., 1995). As noted in the introduction, the glycine ligands evaluated have all shown activity in vivo upon systemic administration. Anxiolytic effects (Anthony and Nevins, 1993; Trullas et al., 1991), antidepressant effects (Trullas and Skolnick, 1990), anticonvulsant activity (Singh et al., 1990a; Skolnick et al., 1989; Witkin and Tortella, 1991; Peterson and Schwade, 1993), effects on unconditioned behavior (Witkin, 1993) and effects on memory (see Witkin, 1995 for an overview) have all been reported to occur with these compounds in the range of doses administered in the present study. D-serine, although administered directly into the brain, neither substituted for nor otherwise altered the discriminative stimulus effects of PCP. After systemic administration in the doses and times of testing in the present study, significant levels of ACPC are achieved in the central nervous system (Miller et al., 1992). The use of direct central injections, the availability of in vivo indicators of NMDA antagonism (e.g., anticonvulsant actions), and direct assessments of central nervous system uptake, suggest that the lack of behavioral side-effects (including their discriminative stimulus effects) of these glycine-site ligands is not due to their generally poor penetration into brain after systemic administration.

In the report by Contreras (1990), D-serine completely and dose-dependently (0.1–1 μmol/rat i.c.v) prevented the ataxia and stereotypy produced by PCP. In contrast, in the reports by Tanii and colleagues, blockade of ataxia, locomotor stimulation and stereotypy was incomplete across a somewhat higher range of doses of D-serine (up to 2 μmol/rat i.c.v). Moreover, both D-serine and D-alanine produced behavioral

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effects at the doses that blocked effects of PCP (Tanii et al., 1994). For example, locomotor-stimulant effects of PCP were reduced only at doses of the amino acids which decreased locomotor activity when given alone. Tanii et al. (1994) also acknowledged the possibility that the amino acids attenuated the effects of PCP through actions other than at the strychnine-insensitive glycine site. In the present study, i.e., in vivo, administration of d-serine up to 2 μmol/rat was without effect on the discriminative stimulus effects of PCP. D-Serine also did not substitute for PCP. The lack of blockade was not caused by the particular training dose of PCP because d-serine also did not alter the dose-effect function for the discriminative stimulus effects of PCP. Nonetheless, rates of responding in the presence of 2 μmol d-serine were lower than with vehicle pretreatments which indicated biological activity at the doses tested. The inability of d-serine to alter the effects of PCP in the present study suggests that either not all of the effects of PCP can be blocked by glycine agonists (motor us. discriminative effects) or that the blockade by these compounds is not a robust phenomenon. Moreton et al. (1991) have shown that d-serine also did not alter the electroencephalographic effects of PCP or PCP-induced ataxia. With few exceptions, functional antagonists of the strychnine-insensitive glycine receptor produce important pharmacological actions (e.g., anti-ischemic, anticonvulsant) and behavioral effects (e.g., anxiolytic, antidepressant) without inducing the psychotomimetic effects which characterize PCP and related NMDA antagonists or the sedative effects of competitive NMDA antagonists. The results of the present study indicate that this pharmacological profile may also extend to the subjective effects of this class of compounds. As a model predictive of the subjective effects of drugs in humans (Balster and Willetts, 1988; Schuster and Johanson, 1988; Holtzman, 1990; Kamien et al., 1993), the discriminative stimulus effects of strychnine-insensitive glycine receptor ligands suggest that this class of compounds does not have the same liability for producing PCP-like subjective effects. Ultimate confirmation of this prediction must be gained from controlled clinical evaluation. In this regard, it must be noted that some NMDA antagonists that fully substitute for the discriminative stimulus effects of PCP have been shown to be safe therapeutic agents. The low-affinity, uncompetitive NMDA antagonist, memantine, for example, has been shown to be safe therapeutic agents. The low-affinity, uncompetitive NMDA antagonist, memantine, for example, substitutes for the discriminative stimulus effects of PCP in rats (Sanger et al., 1992) and for dizocilpine in mice (Geter-Douglass and Witkin, in press) and yet did not show PCP side effects in the clinical treatment of dementia or Parkinsonism (cf. Ditzer, 1991; Rabey et al., 1992). The results of the present study, in conjunction with a growing body of preclinical efficacy studies, are encouraging about the possibility that functional antagonists of the NMDA-associated glycine site may be valuable therapeutic entities for disorders involving NMDA hyperfunction.

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