Effects of (+)-HA-966, CGS-19755, Phencyclidine, and Dizocilpine on Repeated Acquisition of Response Chains in Pigeons: Systemic Manipulation of Central Glycine Sites$^{1,2}$

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Accepted for publication December 6, 1998 This paper is available online at http://www.jpet.org

ABSTRACT

The effects of i.m. injections of (+)-HA-966, a glycine-site antagonist at the N-methyl-D-aspartate (NMDA) subtype of the glutamate receptor, its enantiomer (-)-HA-966, the competitive glutamate antagonist CGS-19755, the uncompetitive glutamate antagonists phencyclidine and dizocilpine, and the μ opioid agonist morphine were evaluated in a repeated acquisition task in pigeons. All of the drugs produced dose-dependent decreases in rates of responding. The NMDA receptor and channel blockers and (+)-HA-966 appeared to have a greater effect on acquisition than did morphine at doses that did not fully suppress responding. The rate suppression and learning impairment produced by a large dose of (+)-HA-966 (100 mg/kg) were completely prevented by coadministration of the glycine-site agonist D-serine (560 mg/kg) but not by its enantiomer, L-serine (1000 mg/kg). D-Serine, however, produced incomplete antagonism of the effects of dizocilpine and phencyclidine and failed to alter those of CGS-19755. These findings provide evidence that reducing the activity of the NMDA subtype of the glutamate receptor through pharmacological action at any of three sites produces similar decrements in acquisition, and those produced through antagonism of the glycine site are differentially sensitive to the glycine-site agonist D-serine.

Activation of the N-methyl-D-aspartate (NMDA) subtype of glutamate receptors requires the presence of glycine at a site on the receptor that is distinct from the site at which NMDA and glutamate bind (Corsi et al., 1996). The actions of glycine at this site are not blocked by strychnine, which makes them distinct from the inhibitory actions of glycine on glycine-sensitive chloride channels (Vannier and Triller, 1997). There are several similarities between compounds such as (+)-3-amino-1-hydroxy-2-pyrrolidine (HA-966) and 7-chlorokynurenic acid (7CKA), which block binding of glycine at its site on this glutamate receptor; compounds such as cis-4-phosphonomethyl-2-piperidine carboxylic acid (CGS-19755) and DL-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid, which are competitive antagonists at the glutamate binding site; and noncompetitive antagonists such as phencyclidine and dizocilpine, which block the ion channel of the NMDA receptor. All, for example, have protective effects against the effects of central hypoxia and ischemia (McDonald et al., 1989; McNamara and Dingledine, 1990; Priestley et al., 1990; Wood et al., 1992), and all have anticonvulsant effects (Croucher and Bradford, 1990, 1991; Singh et al., 1990; McDonald et al., 1992). The fact that the anticonvulsant effects of (+)-HA-966 and 7CKA are mediated through the glycine site was demonstrated by the ability of i.c.v. administration of the glycine-site agonist D-serine to prevent the anticonvulsant effects of these antagonists but not those of an antagonist at the glutamate site (Lu, 1994). A third common aspect is that both the glutamate site and the glycine site on the NMDA receptor have been implicated in learning and long-term memory. Morris et al. (1986) found that i.c.v. administration of aminophosphonovaric acid, a competitive antagonist at the NMDA site, selectively impaired the ability of the rats to acquire spatial information and prevented the induction of hippocampal long-term potentiation, which may be associated with synaptic plasticity relevant to acquisition of new behavior. The competitive glutamate-site antagonists DL-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid and DL-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid carboxy-ethylster have also been reported to cause decrements in the performance of rats in a radial arm maze (Butelman, 1989; Bischoff and Tiedtke, 1992). In operant repeated acquisition

ABBREVIATIONS: CGS-19755, cis-4-phosphonomethyl-2-piperidine carboxylic acid; 7CKA, 7-chlorokynurenic acid; HA-966, 3-amino-1-hydroxy-2-pyrrolidine; NMDA, N-methyl-D-aspartate.
procedures, the unconvergent glutamate antagonists phenylalnine and dizocilpine impaired acquisition in rats (Cohn and Cory-Slechta, 1992), monkeys (Moerschbaecher et al., 1985; France et al., 1991), and pigeons (Thompson and Moerschbaecher, 1982). Recent reports have identified learning deficits produced by glycine-site antagonists. Intracorony administration of 7C4A blocked the ability of chicks to learn passive avoidance (Steele and Stewart, 1993). This same glycine-site antagonist impaired the working memory of rats in a three-runway task, and this impairment was prevented by the intrahippocampal administration of n-serine (Ohno et al., 1994). The present experiments were designed to compare the glycine-site antagonist (+)-HA-966 with the glutamate-site competitive antagonist CGS-19755 and the uncompetitive antagonists dizocilpine and phenycyclidine with respect to their ability to modify complex behavior in pigeons after parenteral (i.m.) administration. A repeated acquisition task was used, similar to those used in previous studies, to evaluate drug interactions with learning and memory. In addition, the ability of parenteral administration of the glycine-site agonist n-serine to reverse the deficits produced by each of these antagonists was evaluated to determine whether their similar effects on acquisition and performance were mediated through a similar or different site.

Materials and Methods

Subjects. Eight experimentally naive White Carneau pigeons (Palmetto, Sumter, SC), maintained at approximately 80% of their free-feeding weight, constituted the study population. The pigeons were individually housed in cages, with water and grit freely available, in a colony room with a 12-h light/dark schedule (lights on at 7:00 a.m.). All animals had previously received phenycyclidine-like compounds. Animals in these studies were maintained in accordance with the University Committee on the Use and Care of Animals, University of Michigan and “Guidelines of the Committee on the Care and Use of Laboratory Animals” of the Institute of Laboratory Animal Resources, National Health Council (Department of Health, Education and Welfare, publication no. NIH 85-23, revised 1983).

Apparatus. Experiments were conducted in ventilated, sound-attenuating chambers measuring 36 × 28 × 33 cm. Operant conditioning boxes were located within each chamber and had three translucent response keys, 2.4 cm in diameter, located on the middle of one wall, 25 cm from the floor. The keys were 5 cm apart and could be transilluminated by blue, red, green, or amber (red and green lights simultaneously illuminated) 7-W lights located behind the wall. Mixed grain was made available by means of a hopper that could be raised to a position below the center response key and 10 cm from the floor of the chamber. During food availability, the hopper was illuminated by a 7-W white light. Execution of the experiments and data collection were accomplished using an IBM model 70 PC and Med-State software (Med Associates, Inc., East Fairfield, VT).

Repeated Acquisition Procedure. Pigeons were trained to acquire a four-link response chain under a second-order FR5 schedule (Thompson, 1973). The terminal schedule was one in which all three keys were illuminated red at the beginning of a trial. The pigeon could respond on any of the three keys but only one was designated “correct.” A response on that key turned all three keys green. A peck on one of the two “incorrect” red keys resulted in a 4-s timeout during which the chamber was dark and key pecks had no programmed consequences. After the timeout, the three lights were again illuminated red; the same key was designated correct, and a response on this key turned all the keys green. When all keys were green, a peck on the one correct green key turned all the keys amber. A peck on one of the two incorrect keys darkened the chamber for 4 s, and then the three green keys were again illuminated. In the presence of amber key lights, a correct response turned all the keys blue and a correct response in the presence of the blue key lights produced a 0.5-s flash of the hopper light and restarted the cycle in red. On the fifth time through the cycle (FR5), a correct response in the presence of the blue light resulted in 4-s access to mixed grain. The sequence of light colors was constant (red, green, amber, blue) each day. However, the key that was designated correct in the presence of each light color was changed from day to day, and the response-sequence made by the pigeon had to be altered each day and match the newly programmed key sequence to produce food.

Training the pigeons on this schedule was initiated by illuminating the center key blue and presenting food as a consequence of a peck on this key. Once the key peck response had been made and food presented, all three keys were illuminated blue, and the pigeon was required to peck the correct key to produce access to grain. Incorrect selection resulted in a 4-s timeout followed by reillumination of the blue key lights. Once the pigeon had pecked the correct blue key, the training was continued by illuminating all keys amber and reinforcing correct amber key selection by turning the keys blue. The key that had been correct during previous exposure to the blue lights was still correct, and a response on this key resulted in grain presentation. The training continued to step backward in the key color presentation until a correct response in the presence of the red key lights turned all keys green, a correct response in the presence of the green key lights turned all the keys amber, and a correct response in the presence of the amber key lights turned all the keys blue. A correct response in the presence of the blue key light resulted in grain presentation during the initial steps of training; the number of times the pigeon was required to complete the four-color links in the chain was gradually increased to five. Incorrect responses in any key color produced a 4-s timeout and the subsequent reillumination of three keys in the color in which the error had been made.

Sessions were typically run 6 days per week. Each session began with a blackout period lasting approximately 5 min. During this blackout period, key pecks had no programmed consequence. Sessions terminated after pigeons received 60 reinforcers or 60 min had elapsed. Correct key locations for each link color were changed on a daily basis according to a schedule of three sets of six series (18 distinct orders). The series were selected to be equivalent in several ways with restrictions in their ordering across sessions (Thompson, 1973). An example of a set of six chains is LRCR, CLRL, LRLC, RCLR, CLCR, and RCLC, where L is left, R is right, and C is center. For all chains, the first response requirement was in the presence of red stimulus lights, the second was in the presence of green stimulus lights, the third was in the presence of amber stimulus lights, and the last was in the presence of blue stimulus lights.

Pigeons were trained until a steady state of acquisition was reached. This required that animals consistently receive at least 54 (90%) of the available 60 food reinforcers while responding more rapidly than 0.5 responses/s, with a maximum of 33% errors during the entire session. (Errors were designated as the number of incorrect key pecks divided by the total number of key pecks multiplied by 100 and are referred to as percent errors.) When an animal achieved this level of acquisition, baseline data were collected for 8 to 10 days, and mean values were calculated for the percent errors and response rate.

Drug evaluation was initiated when criteria were met for 5 consecutive days. The error criterion was that the animal make no more or fewer errors than 15% of its baseline error number. To meet the response rate criterion, the number 0.18 was added and subtracted from the session’s response rate, and the established baseline rate had to fall within these values. The 0.18 value is 15% of the typical high rate in this procedure of 1.2 responses/s. After each drug test, criterion-level performance had to be reached for 2 consecutive days before subsequent testing.

Design. Four or five pigeons were tested with each drug and with saline. All drugs were administered in a single i.m. injection in a
Results

Pigeons typically reached criterion-level performance of the repeated acquisition procedure after 6 to 8 months of training. Values of baseline percent errors and response rates were very similar across animals. In baseline sessions, most errors were made as the first few reinforcers were earned (i.e., reinforcers 1–3, or 5–15 correct sequences).

Comparison of NMDA Antagonists. A detailed illustration of the effects of the glycine-site NMDA antagonist (+)-HA-966 on accuracy and rate of responding can be observed over the first 28 reinforcers earned within a session by a single animal (Fig. 1). Although each dose caused this pigeon to make more errors compared with saline control (Fig. 1, top), only 56 mg/kg was associated with a large reduction in response rate (Fig. 1, bottom). Dose of both 10 and 32 mg/kg had modest response rate-reducing effects that were restricted to the early portion of the session (Fig. 1, bottom). After the administration of 10 mg/kg (+)-HA-966, error accumulation declined by the 28th reinforcer (Fig. 1, top). This is evident by the fact that only two errors were made in earning the 24th through 28th reinforcer, indicating that the sequence was probably acquired at this point in the session. In contrast, the animal made 23 errors in earning the 24th through 28th reinforcer after the administration of 32 mg/kg (+)-HA-966. Similarly, no reduction in error accumulation was observed after the administration of 56 mg/kg (+)-HA-966; the animal stopped responding after making 174 errors, earning six reinforcers. Thus, no evidence of acquisition of the sequence was observed after the administration of 32 or 56 mg/kg (+)-HA-966. Although this animal’s rate of responding was different with each dose of (+)-HA-966 for the first six reinforcers, cumulative errors per reinforcer for the first six reinforcers were quite similar across doses.

Data representing the effects of the enantiomers of HA-966 on percent errors and response rates for each of five birds and their average in the repeated acquisition program are shown in Fig. 2. Both enantiomers produced dose-dependent increases in percent errors and decreases in rates of responding. The (+)-enantiomer was more potent in decreasing rates of responding (Fig. 2, right bottom) and in increasing errors (Fig 2, right top). There were substantial individual differences among the pigeons with respect to their response to the HA-966 enantiomers, but there was a general association between decreased rates of responding and increased percent errors for the individual animals for both enantiomers.

In the repeated acquisition schedule, each of the three NMDA antagonists, dizocilpine, phencyclidine, and CGS-19755, as well as the μ opioid morphine, produced dose-dependent increases in percent errors and reduced response rates (Fig. 3). Morphine, however, produced fewer errors at rate-decreasing doses, suggesting less effect of this drug on acquisition behavior. At doses that produced a 50% decrease in rates of responding, dizocilpine produced 62% errors, phencyclidine produced 39% errors, and CGS-19755 produced 50% errors. Morphine, in contrast, at a dose that caused a 50% decrease in response rates, produced only 27% errors.

Coadministration of D-Serine With NMDA Antagonists. A dose of 100 mg/kg (+)-HA-966 produced marked
decreases in rates of responding, and marked increases in percent errors, as shown in Fig. 2. Administration of increasing doses of D-serine produced dose-dependent reversals in the percent errors and response rate reductions caused by administration of 100 mg/kg (+)-HA-966 (Fig. 4, left). At the largest dose of D-serine (560 mg/kg) coadministered with 100 mg/kg (+)-HA-966, the mean percent errors (Fig. 4, left top) and response rates (Fig. 4, left bottom) were not substantially different from saline control values. This dose of D-serine administered alone had no effect on percent errors but slightly reduced response rates. Unlike D-serine, L-serine did not completely reverse the effects of (+)-HA-966 on percent errors and response rates in the repeated acquisition procedure (Fig. 4, right). Although percent errors after the coadministration of 1000 mg/kg L-serine with 100 mg/kg (+)-HA-966 was moderately lower than with 100 mg/kg (+)-HA-966 alone, this value was still much greater than saline control (Fig. 4, right top). In addition, L-serine only slightly reversed the profound reduction in response rates caused by 100 mg/kg (+)-HA-966 (Fig. 4, right bottom). Thus, the reversal by D-serine of the behavioral effects caused by (+)-HA-966 was stereoselective.

A detailed illustration of the reversal by D-serine of the effects caused by 100 mg/kg (+)-HA-966 on accuracy and rate of responding in the repeated acquisition procedure is shown over the first 28 reinforcers averaged over all five animals (Fig. 5). The largest doses of D-serine (560 mg/kg) restored both percent errors and response rates to those observed after saline administration. The next smallest dose of D-serine (320 mg/kg) also returned response rates to predrug baseline levels (Fig. 5, bottom) but did not greatly modify the rate of error accumulation compared with administration of 100 mg/kg (+)-HA-966 alone (Fig. 5, top).

Administration of a range of doses of D-serine along with a dose of dizocilpine (180 mg/kg) that produced marked increases in percent errors and decreases in response rates resulted in the partial attenuation of the dizocilpine-induced impairments (Fig 6). The largest dose of D-serine (560 mg/kg) was most effective in this regard (Fig. 6, left). These effects, although modest and with considerable interanimal variability, were reliably observed within animals over three determinations per animal. The two smaller doses of D-serine (100, 320 mg/kg) had very little effect on the dizocilpine-induced impairments. Coadministration of 1000 mg/kg L-serine with 180 mg/kg dizocilpine did not modify the effects of dizocilpine (Fig. 6, right). D-Serine was less effective in reversing deficits produced by another NMDA channel blocker, phencyclidine (1.8 mg/kg) in the repeated acquisition procedure (Fig. 7, left) and entirely ineffective against comparable deficits caused by the competitive NMDA antagonist CGS-19755 (3.2 mg/kg; Fig. 7, right).

**Discussion**

Intramuscular administration of (+)-HA-966, an antagonist at the glycine binding site of the NMDA subtype of the
glutamate receptor, caused a dose-dependent reduction in accuracy of responding and rates of responding in a repeated acquisition task in pigeons. A similar decrement was produced by dizocilpine and phencyclidine, drugs that block the NMDA channel, and by CGS-19755, a competitive antagonist at the glutamate binding site. Although a reduction in response rates was often observed at doses that caused an impairment in acquisition, rate-decreasing effects were not always accompanied by acquisition impairments. Only the largest doses of (−)-HA-966, CGS-19755, phencyclidine, and dizocilpine reduced response rates below the criterion level of 0.5 responses/s. Intermediate doses of each NMDA antagonist did not profoundly affect rates of responding, yet each increased percent errors to greater-than-maximum criterion levels of 33%. The (−)-enantiomer of HA-966, which is relatively less active as a glycine-site antagonist (Pullan et al., 1990; Singh et al., 1990), was 0.5 to 0.75 log unit more potent in suppressing rates of responding, and nearly as effective in blocking acquisition of behavior at doses just below those that stopped all responding. Morphine, which has no clear interaction with NMDA receptor neurotransmission, produced considerably less decrement in acquisition at doses that profoundly suppressed rates, a result similar to that found by Thompson and Moerschbaecher (1981).

These data suggest that blockade of ion transport through the NMDA channel by pharmacological action at any of these three relevant binding sites can produce a very similar reduction of acquisition of learning in a relatively selective manner. Similar conclusions were reached by Xu et al. (1995) in studies of the anterograde amnesia, fear conditioning, and anticonvulsant effects of (−)-HA-966 and dizocilpine. These data also provide further support for the notion that activation of the NMDA subtype of glutamate receptors is an important aspect of at least some types of learning.

It is unlikely that (−)-HA-966 was acting at a site on the NMDA receptor other than the glycine site because systemic administration of D-serine, an agonist at this glycine site,
produced a dose-dependent reversal of both the rate and learning impairments caused by (+)-HA-966. At the largest dose of D-serine, the reversal of (+)-HA-966-induced impairments was complete. When coadministered with an effective dose of the uncompetitive antagonist dizocilpine, the largest dose of D-serine returned both acquisition and rate of responding to approximately 50% of control values. Smaller doses had little effect. Modest improvements in phencyclidine-impaired responding were observed but were not related to D-serine dose, and D-serine had no effect on impairments produced by CGS-19755. This indicates a specific interaction of (+)-HA-966 on the glycine site to produce the decrements in acquisition reported here.

Other investigators have found an interaction between D-serine and glutamate channel blockers. Tanii et al. (1994) reported that i.c.v. administration of D-serine produced a dose-dependent but incomplete reversal of hyperactivity and ataxia induced by phencyclidine in the rat; this reversal could be prevented by administration of 7CKA. This suggests that our observation of D-serine-induced partial antagonism of the antiaquisition effects of phencyclidine and dizocilpine might be a general phenomenon. The mechanism of this
effect is unclear but might involve D-serine-induced increases in the number of NMDA channels that are operational, even in the presence of the channel blockers.

Although all of the NMDA antagonists produced similar impairments in the repeated acquisition procedure, their differential sensitivity to D-serine provides evidence that the learning impairments observed were produced by activity at different ligand-binding sites. Similarly selective pharmacological interactions have been reported previously after the central administration of D-serine. For example, i.c.v. administration of D-serine reversed the inhibition of NMDA-induced convulsions caused by i.c.v. administration of 7CKA and i.c.v. or i.p. administration of (+)-HA-966 in mice, but was ineffective against the anticonvulsant effects of CGS-19755 (Lu, 1994). In pigeons, the response-rate decreasing effects of i.c.v. administration of (+)-HA-966 or 7CKA, but not i.c.v. administration of CGS-19755, were also reversed by i.c.v. administration of D-serine (Lu, 1994).

Pharmacological characterization of the electrophysiological (Johnson and Ascher, 1987; Kushner et al., 1988; Huettner, 1989; Vycklicky et al., 1990) and in vitro binding (Mayer et al., 1989; Lerma et al., 1990; Parsons et al., 1993) characteristics of glycine-site antagonists has demonstrated that these effects are selectively mediated at glycine sites associated with the NMDA receptor. Pharmacological characterization of glycine-site antagonists has not been well established using behavioral measures. In addition, systemic bioavailability of glycine-site antagonists has not been previously demonstrated using subconvulsive doses. The present findings provide robust evidence that central glycine sites can be pharmacologically manipulated by systemic administration of a glycine-site agonist and antagonist and that the effects of blocking the NMDA receptor with a glycine-site antagonist are similar to those produced by blocking this receptor at either of two other sites.

References
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