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ORIGINAL INVESTIGATION

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Competitive and uncompetitive *N*-methyl-D-aspartate antagonist discriminations in pigeons: CGS 19755 and phencyclidine

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Abstract The purpose of the present studies was to examine representative uncompetitive and competitive NMDA antagonists, as well as the glycine/NMDA antagonist, HA 966, in pigeons trained to discriminate either PCP or CGS 19755 from saline. Separate groups of pigeons were trained to discriminate either the uncompetitive, phencyclidine (PCP; 0.32 and 1.0 mg/kg, IM), or the competitive, CGS 19755 (*cis*-4-phosphonomethyl-2-piperidine-carboxylic acid; 1.8 mg/kg, IM), NMDA antagonists from saline. Uncompetitive and competitive NMDA antagonists were examined in generalization studies, as were the racemate and the (+) and (–) stereoisomers of HA 966 (3-amino-1-hydroxypyrrolid-2-one). Dizocilpine (MK 801) was fully generalized to PCP but not to CGS 19755. All competitive NMDA antagonists tested were fully generalized to CGS 19755, but not to PCP. The competitive antagonists, however, produced >50% PCP-appropriate responding. The (+) isomer of HA 966 was fully generalized by three of four pigeons discriminating PCP (1.0 mg/kg) or CGS 19755, whereas the racemate and the (–) isomer produced <40% drug-appropriate responding in either group. Neither NMDA, morphine, nor pentobarbital produced >10% drug-appropriate responding in either discrimination group. The competitive antagonists tended to produce peak drug-appropriate responding at times greater than 60 min after administration, whereas uncompetitive antagonists produced peak drug-appropriate responding at earlier times. HA 966 also had a relatively slow onset of action as compared to PCP. These results suggest that antagonists acting at different modulatory sites of the NMDA receptor complex produce similar, but not identical, discriminative stimuli.

Key words Drug discrimination · NMDA antagonists Phencyclidine · CGS 19755 · Pigeons

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Subjects serving in this study were maintained in accordance with guidelines of the Committee on Care and Use of Laboratory Animal Resources, National Research Council [Department of Health, Education and Welfare (National Institutes of Health)] Publication No. 85–23, revised 1985. Portions of these data were presented at the 14th Annual Meeting of the Society for Neuroscience (Baron et al. 1988).

Introduction

Recently, discriminative stimulus effects of competitive and uncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonists have been investigated in several species. Reports have described various levels of generalization of discriminative effects of NMDA antagonists. One might predict that compounds that act as antagonists at the NMDA receptor complex, regardless of location of modulatory site of action, would produce similar discriminative stimulus effects. However, this result has not necessarily been the case. In animals trained to discriminate phencyclidine-like compounds, competitive antagonists produce full (e.g., Koek et al. 1987), partial (e.g., Tricklebank et al. 1989), or no (e.g., France et al. 1989) generalization.

There have been fewer studies of discrimination tasks based upon competitive NMDA antagonists. Leander (1989) trained pigeons to discriminate the competitive NMDA antagonist CGS 19755 (Lehmann et al. 1988) from saline and reported that both competitive and uncompetitive NMDA antagonists were fully generalized. However, Willetts et al. (1989) reported

that, in rats trained to discriminate another competitive antagonist NPC 12626 [2-amino-4, 5-(1, 2-cyclohexyl)-7-phosphonoheptanoic acid] (Ferkany et al. 1989), a competitive antagonist, CPP [3-(2-carboxypiperazine-4-yl)-propyl-1-phosphonic acid], but not an uncompetitive antagonist, PCP, was generalized to the training cue. In a continuation of the Willetts et al. study, Bobelis and Balster (1993) reported results similar to previous findings: the competitive NMDA antagonists CGS 19755, CPPene (D-3-(2-carboxypiperazin-4-yl)-1-propenyl-1-phosphonic acid), and CGP 37849 (DL-(*E*)-2-amino-4-methyl-5-phosphono-3-pentenoic acid) were generalized to the NPC 12626 training cue, whereas the uncompetitive antagonists dizocilpine and dextromethorphan, up to doses which greatly reduced response rates, were not fully generalized. Similar findings were reported in squirrel monkeys (Gold and Balster 1993). These data, in part, suggest differences between the discriminative effects of NMDA antagonists. Less work has been done to investigate the behavioral actions of compounds which act as antagonists at other modulatory sites on the NMDA receptor-complex. (+)-HA 966 is a glycine/NMDA antagonist which, when tested in rats trained to discriminate PCP from saline, was not generalized, nor was PCP generalized in rats discriminating (+)-HA 966 from saline (Singh et al. 1990). The purpose of the present studies was to examine representative uncompetitive and competitive NMDA antagonists, as well as the glycine/NMDA antagonist HA 966, in pigeons trained to discriminate either PCP or CGS 19755 from saline, in particular, focusing on both dose-effect evaluations and time course studies of their discriminative effects. Two training doses of PCP were originally used to examine the effects of different training doses on generalization patterns (Koek et al. 1987); some data from the continuation of those studies are included here.

Materials and methods

Subjects

Fourteen naive White Carneaux pigeons (Palmetto Pigeon Plant; Sumter, S.C.) were maintained at 85% free-feeding weight with Purina Pigeon Chow supplemented with mixed-grain during experimental sessions. Mineral grit and water were freely available in the home cages. Pigeons were individually housed in a colony room maintained at approximately 72°F with a 16:8-h light:dark cycle.

Apparatus

Experiments were conducted in ventilated, sound-attenuated operant chambers. Each chamber contained three 2.5 cm diameter response keys, located 5 cm apart, and 22 cm above the chamber floor. The keys could be transilluminated by 7-W lights directly behind each key. Mixed grain was made available via a 5 × 5 cm opening located directly below the center key. Experiments were controlled by IBM PCjr microcomputers, using BASIC programming language, located in the room adjacent to the experimental chambers.

Initial shaping

All pigeons were initially trained to respond on a single, red-illuminated key. Subsequently, 20 responses on a single key were required to produce food presentation. Four pigeons were trained to discriminate intramuscular injections of 0.32 mg/kg PCP from saline and four were trained to discriminate 1.0 mg/kg PCP from saline. Six pigeons were trained to discriminate IM injections of 1.8 mg/kg CGS 19755 from saline.

Discrimination training

PCP

After naive subjects were trained on a FR20 schedule of food-reinforced responding on a single key, PCP and saline training commenced. Initially, either IM PCP or saline was administered and subjects were placed in the experimental chambers. Four pigeons were trained to discriminate 0.32 mg/kg PCP from saline using previously described procedures (Koek et al. 1987). In the other four pigeons, doses of PCP (0.32 or 0.56 mg/kg) lower than the ultimate training dose of 1.0 mg/kg were administered because this dose (1.0 mg/kg) initially reduced rates of responding. Lower doses were administered for several sessions until rates of responding appeared stable. The PCP dose was then increased to 1.0 mg/kg. A 10-min time-out (TO) period, in which no keys were lit and responses had no scheduled consequence, preceded a 30-min response period in which a single key was lit: left key after PCP injections and right key after saline injections. During the response period, 20 responses on the lit key resulted in food presentation (4-s access to mixed grain), with 32 food-presentations available per session. Drug and saline training sessions were alternated daily as follows: S-D-S-D-S-S-D-D, where S=saline, and D=drug. After eight sessions in which a single key was lit (four PCP training sessions, and four saline training sessions), both keys were illuminated and training continued until pigeons met criterion for nine of ten consecutive training sessions. Criterion was defined as fewer than 20 responses on the injection-inappropriate key prior to the first food presentation and ≥ 90% of total responses emitted on the injection-appropriate key. Subsequent test sessions were conducted after at least two consecutive training sessions in which criterion was met (one under drug and one under saline training conditions). Sessions were conducted 6 days per week. In experiments in which pigeons were trained to discriminate 0.32 mg/kg PCP, training conditions were the same as for the 1.0 mg/kg PCP training group. In these subjects compounds from several pharmacological classes were tested. Subsequently, the training dose was increased to 1.0 mg/kg (approximate re-training time was 1 month); the group of pigeons trained to discriminate 1.0 mg/kg PCP from saline therefore included the four pigeons originally trained to discriminate 0.32 mg/kg PCP from saline and pigeons trained to discriminate 1.0 mg/kg PCP, as described in previous sections.

CGS 19755

The training procedure was similar to the training of the PCP discrimination. Pigeons were initially trained to a FR20 schedule of food-reinforced responding on a single key. A dose of 1.8 mg/kg IM CGS 19755 was chosen as one which approached cataleptic doses, but did not produce catalepsy in pigeons (Lu et al. 1992). CGS 19755 was administered IM and subjects were returned to

their home cages for 80 min. Pigeons were then placed in the experimental chambers. Each session began with a 10-min time-out in which no keys were lit, followed by a 30-min response period with 32 food presentations available. This schedule resulted in a total of a 90-min treatment time after administration of CGS 19755 prior to the start of response periods. This time was chosen a priori based upon the onset of action of the cataleptic effects of CGS 19755, in which peak cataleptic effects did not occur until 90 min after administration (Lu et al. 1992). Otherwise, the training schedule for four pigeons continued as in the PCP discrimination task described above. The two remaining pigeons were trained under a similar schedule; however, this schedule did not include training sessions in which only a single key was illuminated in order to make preliminary comparisons between training conditions.

Testing

Generalization tests could be conducted using either a single 30-min response period in which conditions were the same as in the training session, except that 20 responses on either key resulted in access to mixed grain, or using a multiple-cycle test session employed for a cumulative-dosing procedure and time course determinations. Each cycle consisted of either a 10- or 20-min TO and a 5-min response period in which both keys were illuminated and 20 responses on either key resulted in 4-s access to mixed grain. Each response period ended after 5 min or ten food presentations, whichever occurred first. If a pigeon completed ten fixed-ratios in less than 5 min, the keys and houselight were not illuminated and responses had no scheduled consequences. The next cycle would begin at the end of the 5-min response period, regardless of the number of food presentations. The multiple-cycle schedule allowed for a cumulative-dosing schedule or a time sampling analysis of the drug-appropriate responding. For extended time course sampling (e.g., 4 h) subjects were returned to their home cages, with no food available, after completion of 2 h of consecutive multiple cycles. Three hours and 50 min after test drug administration, pigeons were removed from home cages and placed in the experimental chambers. At this time a single 10-min TO/5-min response period cycle followed.

In the case of time course analyses of dizocilpine in PCP-trained pigeons, dizocilpine was injected and subjects placed immediately in the experimental chamber; the first response period began 1 min later. Subsequently, response periods occurred at 15-min intervals after injection of dizocilpine. In pigeons trained to discriminate CGS 19755, dizocilpine was tested under a cumulative dosing procedure with 20-min TO and 5-min response periods (i.e., 25-min cycles).

Drugs

The following drugs were used: phencyclidine hydrochloride (PCP) and TCP (*N*-[1-(2-thienyl)cyclohexyl]piperidine hydrochloride (National Institute on Drug Abuse, Rockville, Md.); *cis*-4-phosphonomethyl-2-piperidine carboxylic acid (CGS 19755; CIBA-Ceigy); 2-amino-5-phosphonovalerate (AP5), 2-amino-7-phosphonoheptanoate (AP7), and *N*-methyl-D-aspartate (NMDA) from Sigma (St Louis, Mo.); 2-amino-4,5-(1,2-cyclohexyl)-7-phosphonoheptanoate (NPC 12626) was a generous gift from J. Willetts, Nova Pharmaceuticals (Baltimore, Md.); *cis*-(+)-4-((2H-tetrazol-5-yl) methyl)piperidine-2-carboxylic acid (LY 233053) was a generous gift from P. Ornstein, Eli Lilly (Indianapolis, Ind.); dizocilpine maleate (MK 801; Merck-Sharpe and Dohme; N.J.); the racemate and the (+) and (–) isomers of 3-amino-1-hydroxypyrolid-2-one (HA 966; National Institute on Drug Abuse, Rockville, Md.); morphine sulfate (Mallinckrodt, St Louis, Mo.); and sodium pentobarbital (Gaines Chemical Works, New York, N.Y.).

PCP, TCP, dizocilpine, and morphine and were dissolved in saline. Pentobarbital was dissolved in a solution of 10% ethanol, 40% propylene glycol, and the remainder distilled water. NMDA, CGS 19755, AP5, AP7, NPC 12626, LY 233053, and HA 966 were dissolved in water and minimal amounts of NaOH, and titrated to neutral pH with lactic acid. All drugs were administered IM (1.0 ml/kg) into the breast muscle.

Data analysis

Data are represented as the mean (± SE) of drug-appropriate (PCP or CGS 19755) responding and the mean (± SE) responses per second. In each discrimination task, dose-response functions for the competitive NMDA antagonists were calculated from data obtained in time course studies; i.e., each data point was chosen from the time (cycle) that a given dose produced the maximal group mean drug-appropriate responding (DAR) during which two or more subjects were responding; the associated rates of responding are also included.

Reported ED50s were calculated using the procedure # 8 of Pharmacological Calculation System of Tallarida and Murray (1987). This procedure does not exclude data points less than 20% or greater than 80% effect levels.

Results

CGS 19755 discrimination

The number of sessions required for pigeons to learn to discriminate 1.8 mg/kg IM CGS 19755 from saline ranged from 21 to 41 sessions (27 ± 4.8; mean ± SE). Two subjects which did not receive injections in association with only the single, injection-appropriate key illuminated learned the discrimination in 24 and 54 training days, respectively. One subject later died for experimentally unrelated reasons; data from five subjects are presented.

CGS 19755 was dose-dependently generalized by all subjects with an ED50 of 0.92 mg/kg (0.74–1.14; 95% confidence limits). When tested over time, the training dose of CGS 19755 (1.8 mg/kg) was fully generalized 90 min after administration. Greater than 90% CGS 19755-appropriate responding was observed 4 h after administration; drug-appropriate responding decreased to below 40% by 8 h after administration (Fig. 1). Saline never produced > 10% CGS 19755-appropriate responding over 8 h (Fig. 1). The training dose of PCP (1.0 mg/kg) was tested over time as a training-dose cross-generalization test in pigeons trained to discriminate CGS 19755. Peak effects of 1.0 mg/kg PCP were obtained 15–30 min after administration, but the group mean of drug-appropriate responding was never greater than 35% (Fig. 1). CGS 19755-like responding produced by PCP did not occur at times after 60 min after administration.

Generalizations

Dizocilpine produced no CGS 19755-like responding up to doses which completely suppressed responding

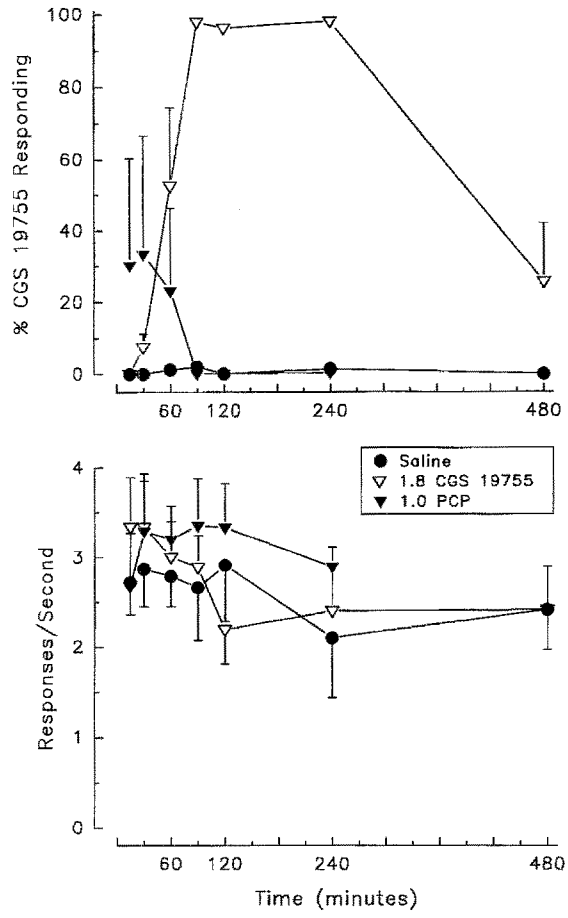

Fig. 1 *Upper panel* presents percentage of CGS 19755-appropriate responding and lower panel response rates (responses/s) after administration of 1.8 mg/kg CGS 19755 (▽), 1.0 mg/kg PCP (▼), and 1 ml/kg saline (●) as a function of time (min) in pigeons trained to discriminate CGS 19755 from saline. Each point represents the mean (±SE) of observations in five pigeons

(Fig. 2). Greater than 50% DAR was produced by PCP only at doses at which three subjects failed to respond (Fig. 2). All competitive NMDA antagonists were dose-dependently generalized; each produced group means greater than 90% DAR. However, NPC 12626, AP7, and LY 233053 produced ≥90% DAR at doses at which one subject did not respond (Fig. 2 and Table 1). AP7 was generalized in three subjects; the fourth pigeon did not respond at doses and times at which other pigeons generalized AP7.

Neither the racemate, nor the (–) isomer of the glycine/NMDA antagonist HA 966, produced greater than 35% drug-appropriate responding (Fig. 2). Each compound was generalized in one of four subjects (Table 1). (+)-HA 966 produced greater than 80% DAR at a dose of 100.0 mg/kg (Fig. 2) and was fully generalized in three of four subjects tested (Table 1).

Neither NMDA, morphine, nor pentobarbital produced greater than 5% DAR (Table I) when examined in the CGS 19755-trained pigeons.

Time course

Times of peak effect of drug-appropriate responding for various compounds were determined in the following manner: the cycle (e.g., cycle 3 = 45 min) during which the group mean of drug-appropriate responding was largest was selected as the time of peak effect of that particular dose. The corresponding rates of responding during that cycle would also be reported. For example, if in three cycles the group mean DAR values were 10%, 85%, and 78%, respectively, and the group mean response rates were 2.5, 1.8, and 1.9

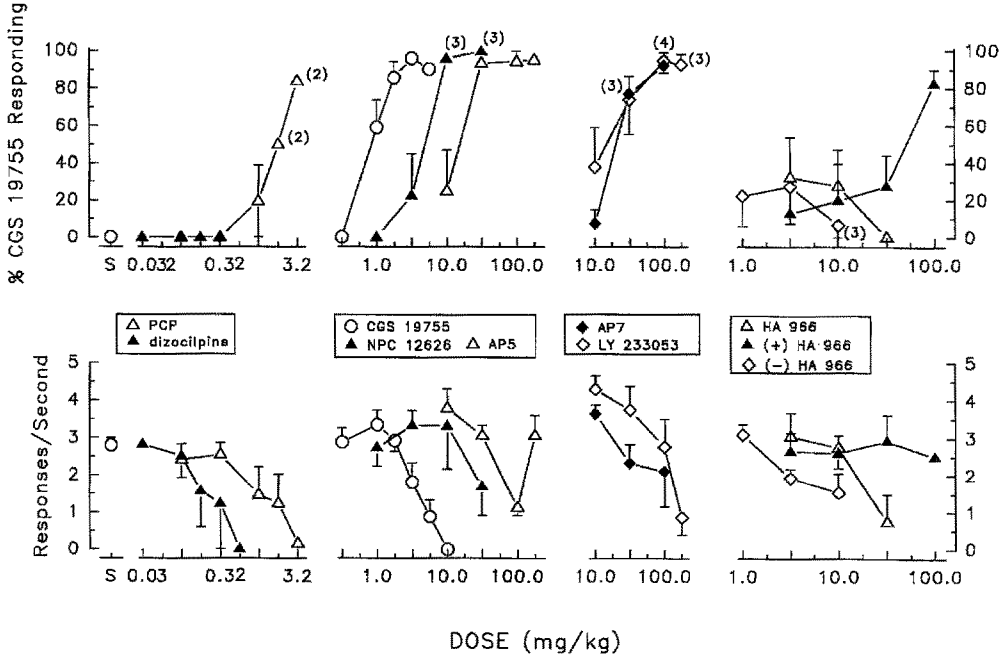

Fig. 2 *Upper panel,* present percentage of CGS 19755-appropriate responding and lower panels present response rates (responses/s) after administration of various NMDA antagonists as a function of dose (mg/kg) in pigeons trained to discriminate 1.8 mg/kg CGS 19755. Points over "S" represent responding after administration of saline. Numbers in parentheses in upper panels represent the number of subjects responding at rates >0.2 responses/s; mean CGS 19755-appropriate responding is calculated using data from these subjects only

Table 1 Summary of time-course cross-generalization tests in the CGS 19755 (1.8 mg/kg) discrimination. N.D. not determinable from data as maximal DAR was 0%. N.A. not applicable as time course data were not determined for NMDA, morphine, or pentobarbital

Drug	Dose range tested mg/kg	Subst *n*/*N*[a]	Max. % dar dose[b]	Max % dar[c]	Rate R/s[d]	Time[e]	Max. rate[f]	Time[g]
NPC 12626	1–32	4/4	32	99.5 (0.5)	1.7 (0.8)	75	0.74 (0.5)	120
AP5	10–180	4/4	32	93.3[h] (3)	3.1 (0.3)	75	1.6 (0.7)	120
AP7	10–100	3/4*	100	92.2 (4)	2.1 (1)	45	0.3 (0.3)	120
LY233053	10–180	5/5	100	94.7 (4)	2.7 (0.7)	30	0.9 (0.5)	75
HA 966	3.2–32	1/4	3.2	32.5 (21)	3.1 (0.6)	105	0 (0)	90
(−)HA 966	1–10	1/4	10	27.5 (20)	1.9 (0.3)	75	0.1 (0.1)	90
(+)HA 966	3.2–100	3/4	100	82.5 (7.5)	2.5 (0.1)	60	1.4 (0.6)	120
NMDA	1–10	0/3	N.D.	0 (0)	N.D.	N.A.	0 (0)	N.A.
Morphine	0.1–10	0/3	3.2	1.1 (0.9)	2.1 (0.4)	N.A.	0 (0)	N.A.
Pentobarbital	1–18	0/3	N.D.	0 (0)	N.D.	N.A.	2.12 (1.1)	N.A.

[a] Number (*n*) of pigeons emitting ≥ 90% DAR at any dose/number (*N*) pigeons tested
[b] Dose which produced maximum mean % CGS 19755 appropriate responding
[c] Maximum % CGS 19755 appropriate responding
[d] Responses per second over the 5-min response periods obtained after administration of the dose resulting in maximum % DAR
[e] Time (minutes after test drug administration) at which pigeons emitted maximum %DAR
[f] Lowest rate of responding obtained at the maximum dose tested
[g] Time (minutes after test drug administration) at which pigeons emitted lowst rates of responding
[h] AP5 180 mg/kg produced slightly higher %DAR than did 32 mg/kg; however rates of responding were later (15–30 min) significantly decreased

responses/s the data obtained during the second cycle would be reported in the dose-response function, i.e., 85% DAR and 1.8 responses/s. Thus, dose-response functions for each test compound for both DAR and rates of responding were composed from data from the cycle in which peak group mean DAR effect was obtained. By using this method, values obtained for each point in a given dose-response curve do not necessarily correspond to the same times after administration. However, corresponding DAR and response rates at any given dose were taken from the same cycle. Tables 1 and 2 contain the times of peak effect of the dose of test compound which produced the group-maximum level of DAR. AP5 (Fig. 3) produced maximum group mean CGS 19755-like responding 75 min after administration, whereas AP7 and LY 233053 produced maximum effects 45 and 30 min after administration, respectively (Table 1). Each competitive NMDA antagonist greatly reduced rates of responding at the maximum dose tested; NPC 12626, AP5, and AP7 suppressed rates 2 h after administration, whereas LY 233053 caused the greatest reduction of rates of responding after 75 min (Table 1). (+)-HA 966 produced maximal DAR 60 min after administration; the same dose also produced the greatest rate reduction after 120 min (Fig. 3).

PCP discrimination

Training dose – 0.32 mg/kg

Using a cumulative dosing procedure, only uncompetitive NMDA antagonists were fully generalized in pigeons trained to discriminate 0.32 mg/kg PCP from saline (Table 2). Neither morphine nor pentobarbital was generalized in any subject. Discriminative stimulus effects of the competitive NMDA antagonists AP5 and CGS 19755 were examined over time (30–105 min after administration) using the multiple-cycle testing procedure. AP5 was generalized in three of four subjects and CGS 19755 was generalized in three of six subjects (Table 3). Maximum mean PCP-appropriate responding produced by AP5 and CGS 19755 was obtained 105 and 90 min after injections, respectively. Response rates were suppressed by the largest doses 90 min after injection of AP5, and 75 min after injection of CGS 19755.

Training dose – 1.0 mg/kg

Using a cumulative dosing procedure, both PCP and dizocilpine were fully generalized to the PCP training

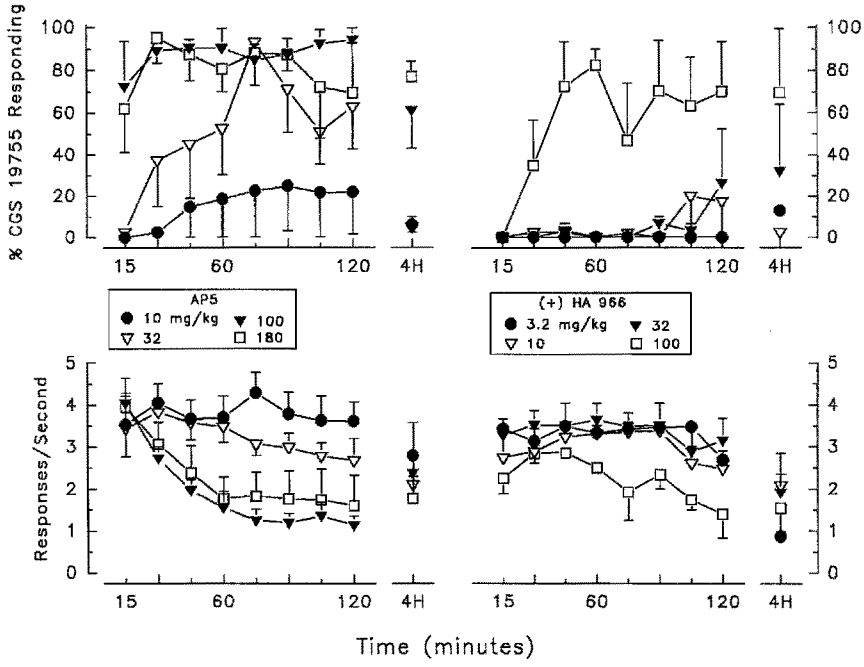

Fig. 3 *Upper panels* present percentage of CGS 19755-appropriate responding and lower panels response rates (responses/s) after administration of various doses of AP5 (*left panels*) and (+)-HA 966 (*right panels*) as a function of time (min) in pigeons trained to discriminate 1.8 mg/kg CGS 19755. Points over "4H" represent responding measured 4 h after administration of the compounds

dose in all pigeons. Dizocilpine was approximately 3-fold more potent in producing discriminative stimulus and rate suppressing effects than PCP (Fig. 4). None of the competitive antagonists produced ≥90% PCP-like responding in all subjects at similar doses and times. CGS 19755 resulted in four of four subjects responding ≥90% DAR; however, this was not necessarily dose-dependent and did not occur at similar times (Fig. 4; Table 3). Nevertheless, three of four pigeons distributed ≥90% of all responses on the drug-appropriate key after administration of AP5 (0.32 mg/kg PCP training dose), AP7, and LY 233053, which produced maximal DAR of 81.9%, 93.1%, and 46%, respectively (Fig. 4 and Table 3). The percent DAR of 93.1 produced by AP7 (100.0 mg/kg) is calculated from data from three pigeons only, as one pigeon did not respond at this time at this dose.

The racemate of the glycine/NMDA antagonist HA 966 was generalized by two of four pigeons and produced a maximal DAR of 32.5%, whereas the (–) isomer was not generalized by any pigeons when administered up to a rate-suppressing dose (Fig. 4 and Table 3). The (+) isomer (100.0 mg/kg) was fully generalized by all pigeons which responded (i.e., three of four), producing a maximum of 96.7% DAR. The apparent order of potency of the racemate and isomers of HA 966 in reducing rates of responding was (–)-HA 966 > (+)-HA 966 > (+)-HA 966.

Table 2 Summary of cross-generalization tests in phencyclidine (0.32 mg/kg) discrimination: N.D. not determined, the test drug did not produce greater than 0% DAR at any dose tested.

Drug	Doses tested[a]	Subst *n*/*N*[b]	Max. % dar dose[c]	Max. % dar[d]	Rate R/S[e]	Rate max dose[f]
PCP	0.1–1.8	6/6	1	95 (2.9)	2.1 (0.4)	0.8 (0.3)
TCP	0.01–1	3/4	0.32	99.7 (0.3)	2.6 (0.5)	0.05 (0.05)
Dizocilpine	0.01–1	5/5	0.32	100 (0)	1.7 (0.2)	0 (0)
Morphine	0.1–1.8	0/4	N.D.	0 (0)	N.D.	0.05 (0.05)
Pentobarbital	1–18	0/4	18	26.4 (14)	0.9 (0.5)	0.9 (0.5)

[a] Dose range tested, mg/kg
[b] Number pigeons (*n*) emitting ≥90% DAR at any dose/number of pigeons (*N*) tested
[c] Dose which produced the maximum mean % DAR
[d] Maximum mean %DAR (numbers in parentheses are one standard error).
[e] Rate of responding (responses/s) over 5-min response period obtained after cumulative-dose administration of the dose resulting in maximum DAR
[f] Rate of responding (responses/s) over 5-min response period at the highest dose tested

Table 3 Summary of time-course cross-generalization tests in phencyclidine (1.0 mg/kg) discrimination. N.D. determined as only 2 subjects responded at this dose at this time

Drug	Dose range tested	Subst *n*/*N*[a]	Max. % dar dose[b]	Max % dar[c]	Rate R/s[d]	Time[e]	Max rate[f]	Time[g]
Dizocilpine	0.1–1.0	4/4	0.32	100	2.14 (0.8)	75	0 (0)	15
CGS 19755	0.1–5.6	4/4	3.2	66.5 (33.2)	1.27 (0.4)	120	0 (0)	90
NPC 12626	3.2–32	2/4	32	54.6 (24)	2.41 (0.7)	60	0.0 (0)	90
AP7	10–100	3/4	100	93.1 (5)	0.9 (.3)	120	0.6 (0.4)	120
LY 233053	10–180	3/4	180	46 (21)	1.83 (0.7)	45	0 (0)	45
HA 966	1–32	2/4	3.2	26.95 (21)	2.8 (0.4)	105	0 (0)	90
(−)HA 966	1–10	0/4	10	8.3 (ND)	0.2 (0.1)	75	0 (0)	90
(+)HA 966	1–100	2/4	3.2	96.7 (3.3)	1.1 (0.6)	90	1.1 (0.6)	90
AP5[h]	1–180	3/4	100	81.9 (ND)	1.6 (ND)	105	0 (0)	90
CGS 19755[h]	0.032–5.6	3/6	3.2	76.2 (12.6)	1.4 (0.4)	90	0 (0)	75

[a] Number (*n*) of pigeons emitting ≥ 90% DAR at any dose/number (*N*) pigeons tested
[b] Dose which produced maximum mean % CGS 19755-appropriate responding
[c] Maximum %CGS 19755-appropriate responding
[d] Responses per second over the 5-min response periods obtained after administration of the dose resulting in maximum %DAR
[e] Time (minutes after test drug administration) at which pigeons emitted maximum %DAR
[f] Lowest rate of responding obtained at the maximum dose tested
[g] Time (minutes after test drug administration) at which pigeons emitted lowest rates of responding
[h] AP5 and CGS 19755 were tested in pigeons trained to discriminate 0.32 mg/kg PCP: time samples were 30 to 105 min after administration of AP5.

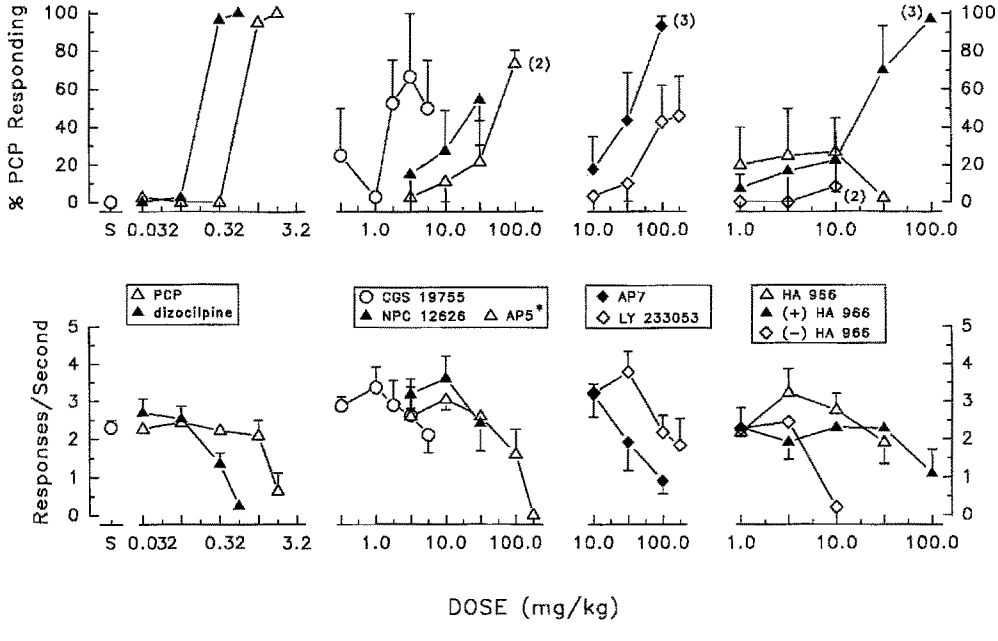

Fig. 4 *Upper panels* present percentage of PCP-appropriate responding and lower panels response rates (responses/s) after administration of various NMDA antagonists, as a function of dose (mg/kg) in pigeons trained to discriminate 1.0 mg/kg PCP. Points over "S" represent responding after administration of saline. Numbers in parentheses in upper panels represent the number of subjects responding at rates >0.2 responses/s; mean PCP-appropriate responding is calculated using data from these subjects only. *AP5 was tested in pigeons trained to discriminate 0.32 mg/kg PCP from saline

Time course

Doses of dizocilpine greater than 0.1 mg/kg produced > 50% PCP-appropriate responding 15 min after administration; however, group peak effect did not occur until 75 min after administration (Fig. 5). When ≥ 50% of subjects responded, peak effect DAR of the maximally effective doses of all the competitive NMDA antagonists, except LY 233053, occurred 60 min or later after administration (Table 3). CGS 19755

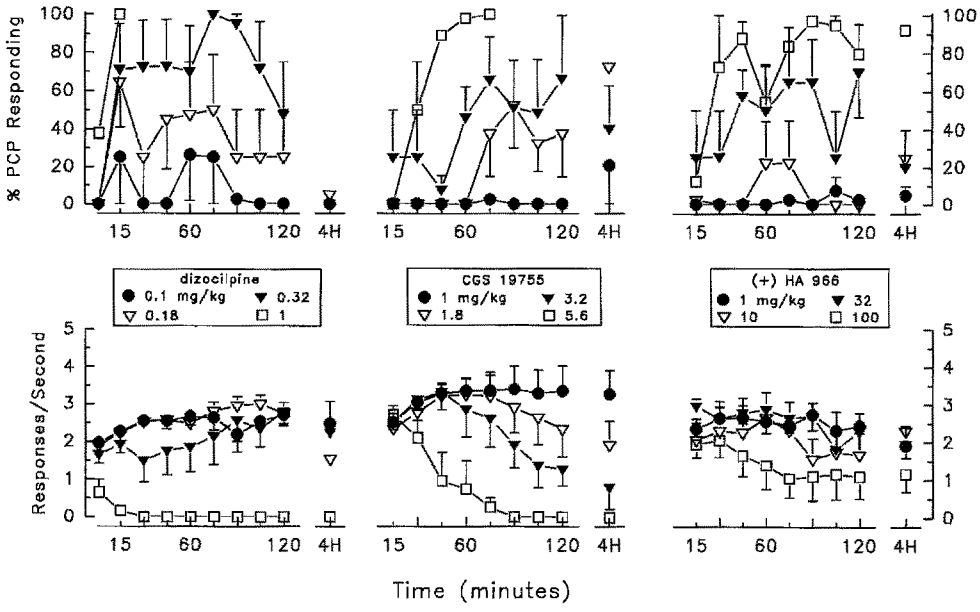

Fig. 5 *Upper panels* present percentage of PCP-appropriate responding and lower panels response rates (responses/s) after administration of various doses of dizocilpine (*left panels*), CGS 19755 (*middle panels*), or (+)-HA 966 (*right panels*), as a function of time (min) in pigeons trained to discriminate 1.0 mg/kg PCP. Points over "4H" represent responding measured 4 h after administration of the compounds

produced peak DAR at times at which rate-decreasing effects were also observed (i.e., ≥ 60 min post-administration, Fig. 5). LY 233053 produced a maximal group mean percent DAR 45 min after administration. Competitive antagonists (excluding AP7) were also tested up to doses that completely suppressed responding; peak DAR effects occurred ≥ 90 min after administration (Table 3). LY 233053 produced a relatively rapid onset of action of rate-suppressing effects, as well, with peak effects occurring 45 min after administration. (+)-HA 966 was generalized in all subjects which responded (i.e., three of four) at a dose of 100.0 mg/kg, with peak effects occurring 90 min after administration. The peak PCP-like effects produced by (+)-HA 966 were also associated with peak rate-decreasing effects (Fig. 5 and Table 3).

Discussion

The competitive NMDA antagonist CGS 19755 (1.8 mg/kg, IM) was readily trained as a discriminative stimulus in pigeons and was dose-dependently generalized as reported previously (Butelman et al. 1993). CGS 19755 produced a discriminative stimulus that was similar to those produced by other competitive antagonists, but could be differentiated from those stimulus effects produced by the uncompetitive antagonists PCP and dizocilpine; PCP and dizocilpine produced <50% or no CGS 19755-appropriate responding, respectively. In contrast, CGS 19755 produced >50% PCP-appropriate responding at doses which did not significantly decrease rates of responding. These results are similar to those reported previously (Willetts et al. 1989; Bobelis and Balster 1993) in which rats were trained to discriminate NPC 12626; CPP, CPPene and CGS 19755 were generalized completely, whereas PCP produced <50% mean drug-appropriate responding. The discriminative stimulus effects of uncompetitive NMDA antagonists have been more widely studied than those of competitive NMDA antagonists (e.g., McMillan and Hardwick 1986; Koek et al. 1987; Jackson and Sanger 1988), and are now widely accepted to be mediated via antagonist activity at the NMDA-type receptors (e.g., Balster and Willetts 1988; Jackson and Sanger 1988; see also Woods et al. 1991). Generally, compounds that act as uncompetitive antagonists produce discriminative stimulus effects to which other uncompetitive antagonists are generalized (e.g. Koek et al. 1988; Willetts and Balster 1988; Tricklebank et al. 1989). In the present study, the uncompetitive NMDA antagonists tested were generalized to the PCP discriminative stimulus, regardless of training dose of PCP. Previously, Koek et al. (1987) examined the effects of PCP, AP5, and metaphit, a proposed PCP-site acylator (Contreras et al. 1986), in separate groups of pigeons trained to discriminate different training doses of PCP from saline. The authors reported that progressively decreasing the training dose of PCP from 1.0 mg/kg to 0.32 mg/kg increased the potencies of the compounds in substituting for the discriminative stimulus without losing selectivity of the discriminative effects. The results shown here present a similar profile of action for the uncompetitive antagonist dizocilpine in the high-or low-dose training conditions. However, CGS 19755 produced slightly different patterns of generalization in pigeons discriminating 0.32 or 1.0 mg/kg PCP. In the low-dose training group CGS 19755 was generalized by three of six subjects tested, with peak DAR effects occurring 90 min after administration and peak rate effects occurring 75 min after dosing, whereas in the high-dose training group, four of four subjects tested generalized to the training cue

with peak DAR and rate-reducing effects occurring 90 and 120 min after administration. However, potencies of drugs in producing peak DAR effects were not changed (Table 3). Although these are not large differences, it may be possible to use different training doses of PCP and/or CGS 19755 to examine differences between the stimulus effects of competitive NMDA antagonists. It is interesting to note that in the present studies the competitive NMDA antagonists presented a somewhat heterogenous profile of PCP-like discriminative effects. CGS 19755 was generalized in all pigeons, though not consistently at the same doses, whereas NPC 12626 was generalized by two of four subjects. It is not clear that these differences can be attributed to different onsets of action as it was observed that LY 233053, a compound with a slightly shorter onset of action, was generalized by three of four subjects tested.

Earlier studies have reported that competitive NMDA antagonists such as CPP are not generalized to stimulus effects produced by PCP or dizocilpine (Jackson and Sanger 1988; Willetts and Balster 1988; Butelman et al. 1991). However, it was not always clear that the times of peak effect of the competitive antagonists were examined within these studies. In rats trained to discriminate PCP from saline, Willetts and Balster administered AP7 IP 30 min prior to testing, and CPP 20 and 60 min prior to testing. The authors also administered AP7 intracerebroventricularly under the assumption that AP7 has poor access to brain when administered parenterally. The study of the onset of action of the cataleptic effects of many competitive antagonists in pigeons has found that, following systemic administration, catalepsy is not observed until 90–120 min after injection (Baron et al 1988; France et al. 1990), whereas central administration produces a rapid onset of catalepsy (Koek et al. 1987; France et al. 1990), suggesting that these compounds indeed have slow access to the brain. These data also suggest that other behavioral effects of these compounds should be examined over broader periods of time.

The present time course studies revealed that the onset of discriminative stimulus effects of NMDA antagonists paralleled that of other behavioral effects produced by the same compounds. The competitive NMDA antagonists produced maximum drug-appropriate responding and rate-decreasing effects in CGS 19755-trained subjects at times which approximated those at which catalepsy occurs under other conditions (e.g., France et al. 1990; Lu et al. 1992). Other competitive antagonists, e.g., NPC 12626, produced similar time courses of action: catalepsy was observed in pigeons 2 h after IM administration and was observed in some subjects after 8 h (France et al. 1990). PCP and dizocilpine produced relatively rapid onsets of discriminative stimulus effects which paralleled those observed for the cataleptic effects of these compounds (onset of 15–20 min; Koek et al. 1986; Leander et al. 1986; Lu et al. 1992). In the present experiments, the time courses of discriminative and rate-reducing effects of competitive antagonists were examined. Generally, after administration of competitive NMDA antagonists, pigeons emitted significant PCP-like responding at times 60 min after injection; high levels of PCP-like responding were also associated with decreased rates of responding. These results are consistent with those found by Butelman et al. (1991), in a study in which pigeons were trained to discriminate dizocilpine from saline: PCP was found to be fully generalized to the training stimulus, whereas CGS 19755 elicited >50% drug-appropriate responding at times later than 1 h and when rates of responding were greatly reduced.

(+)-HA 966, a strychnine-insensitive glycine-site antagonist, has previously been shown to have antagonist action at the NMDA/glycine modulatory site (e.g., Fletcher and Lodge 1988), to be an effective antagonist of NMDA-induced convulsions and lethality (Koek and Colpaert 1990) and has been trained as a discriminative stimulus in rats (Singh et al. 1990). Singh et al. (1990) found that PCP produced little drug-appropriate responding in rats discriminating (+)-HA 966 and that (+)-HA 966 produced a maximum of 33% PCP-appropriate responding. The authors suggested that the differences between the discriminative effects of compounds acting as NMDA antagonists may depend upon the particular mechanism, or site of action, though which the antagonist action is conferred. In addition, the novel glycine-site antagonists ACEA 1011 and ACEA 1021 do not produce PCP-like effects in rats trained to discriminate PCP from saline (Balster et al. 1993). In contrast, the present experiments suggest a greater similarity in the discriminative effects of (+)-HA 966 to PCP and CGS 19755.

In summary, the discriminative stimulus effects of NMDA antagonists acting at different sites within the NMDA-receptor macro-molecular complex were examined in pigeons trained to discriminate competitive (CGS 19755) or uncompetitive (PCP) NMDA antagonists, with particular attention to time course of discriminative effects. The present studies revealed that compounds which act to reduce activity at NMDA receptors elicited a greater percentage of drug-appropriate responding than compounds which act though other pharmacological mechanisms, e.g., morphine and pentobarbital. However, NMDA antagonists acting at different sites of action do not produce identical discriminative stimulus effects.

These are the most extensive studies of the time course of discriminative effects of these compounds to date. The current findings contrast with those of other studies, which have reported no significant generalization produced by uncompetitive antagonists in subjects trained to discriminate competitive antagonists, and vice versa. Earlier investigations tested these compounds at time points which may not have represented times of peak effect. The current

studies indicate that, when examining the behavioral effects, time course of onset and duration of action should be considered.

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