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Decrement in Operant Performance Produced by NMDA Receptor Antagonists in the Rat: Tolerance and Crosstolerance

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DRAVOLINA, O. A., E. E. ZVARTAU AND A. Y. BESPALOV. *Decrement in operant performance produced by NMDA receptor antagonists in the rat: Tolerance and crosstolerance.* PHARMACOL BIOCHEM BEHAV **65**(4) 611–620, 2000.—Current perspectives on the clinical use of NMDA receptor antagonists infer repeated administration schedules for the management of different pathological states. The development of tolerance and crosstolerance between different NMDA receptor antagonists may be an important factor contributing to the clinical efficacy of these drugs. The present study aimed to characterize the development of tolerance and crosstolerance to the ability of various site-selective NMDA receptor antagonists to produce a decrement of operant responding (multiple extinction 9 s fixed-interval 1-s schedule of water reinforcement). Acute administration of D-CPPen (SDZ EAA 494; 1–5.6 mg/kg), dizocilpine (MK-801; 0.03–0.3 mg/kg), memantine (0.3–17 mg/kg), ACEA-1021 (10–56 mg/kg), and eliprodil (1–30 mg/kg) differentially affected operant responding. Both increases and decreases in response rates and accuracy of responding were observed. Repeated preexposure to D-CPPen (5.6 mg/kg, once a day for 7 days) attenuated a behavioral disruption produced by an acute challenge with D-CPPen or ACEA-1021, but potentiated the effects of dizocilpine, memantine, and eliprodil. Based on the present results, one can suggest that the repeated administration of a competitive NMDA receptor antagonist differentially affects the functional activity of various sites on NMDA receptor complex. © 2000 Elsevier Science Inc.

Dizocilpine (MK-801) Memantine D-CPPen (SDZ EAA 494) ACEA-1021 Eliprodil
Operant behavior Tolerance Rats

N-METHYL-D-ASPARTATE (NMDA) receptor is a subtype of excitatory amino acid receptors implicated in a number of physiological functions. A variety of compounds have been designed as antagonists targeting NMDA receptors for a wide range of possible therapeutic applications including brain ischemia, neurodegeneration, and seizure disorders (8,12).

Although perspectives on the clinical use of NMDA receptor antagonists infer repeated administration schedules, experimental evidence is very limited regarding the effects of repeated exposures and the possible development of tolerance and dependence. On one hand, it was shown that phencyclidine (PCP)-like NMDA receptor channel blockers such as dizocilpine (MK-801) produce tolerance and dependence upon repeated administration (2,14,17,28). Repeated administration of NMDA receptor antagonists may also result in sensitization to their pharmacological effects [e.g., (14,20, 36,39)].

Further, the NMDA receptor complex has multiple ligand recognition sites (2,8), and effects of repeated administration may vary significantly among site-selective antagonists. For competitive NMDA receptor antagonists, the development of tolerance was observed to locomotor decreasing [CGS 19755, (4); CGP39551, CGP37849, (21)], cataleptic [CGS 19755, (17)], and motor impairing effects [D-CPPen, (24)]. Nevertheless, several studies reported no tolerance to motor impairing [CGS 19755, (4); CGP 37849, CGP 39551, (10)], anticonvulsant [CGS 19755, (4); CGP 37849, CGP 39551; (10); D-CPPen, (24)], neuroprotective (SDZ 220-581, (26)], anxiolytic [CGP 37849, (13); NPC 17742, (33)] and antidepres-

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sant-like effects [AP-7, (23)]. Very little information exists with regard to the development of tolerance to glycine site and polyamine site antagonists [e.g., tolerance to glycine site partial agonists, (15,23)]. Assuming that different NMDA receptor antagonists may be applied for the clinical management of different pathological states [e.g., see (8)], a single patient may have a history of administration of two or more NMDA receptor antagonists. Therefore, the development of crosstolerance or cross-sensitization between different drugs is of special interest (17,23). For instance, symmetric crosstolerance to cataleptic effects was observed between PCP-like channel blockers and competitive NMDA receptor antagonists (17). Overall, transfer of tolerance between antagonists at different subtypes of the NMDA receptor has not been well studied, and cannot readily be predicted based on the existing knowledge of the NMDA receptor structure. Intrinsic relations between different binding sites are rather complex: administration of a compound selective for one site on the NMDA receptor complex may affect expression and/or binding parameters of other sites [e.g., (6,25)].

The present study aimed to characterize the development of tolerance and crosstolerance to the ability of various site-selective NMDA receptor antagonists to produce decrement of water-reinforced operant responding. Multiple fixed-interval 1-s extinction 9-s schedule of reinforcement was used to enable parallel analysis of the general level of operant performance (rate of lever pressing) and accuracy of responding. Fixed interval (FI) schedules of reinforcement may be used when studying the drugs that produce sensorimotor deficit, and are expected to affect temporal patterns of responding within the FI periods. Preliminary experiments suggested that the accuracy-impairing properties of PCP-like channel blockers (dizocilpine) are best revealed when reinforcer availability is signaled by both time and stimulus lights.

Following the initial determination of dose–effect relationships for various compounds, we examined the development of tolerance to response rate-decreasing effects of a competitive NMDA receptor antagonist, D-CPPen (16). It should also be noted that each of the repeated injections of D-CPPen was delivered after, but not before the daily training sessions and, thus, D-CPPen was likely to result in the nonassociative

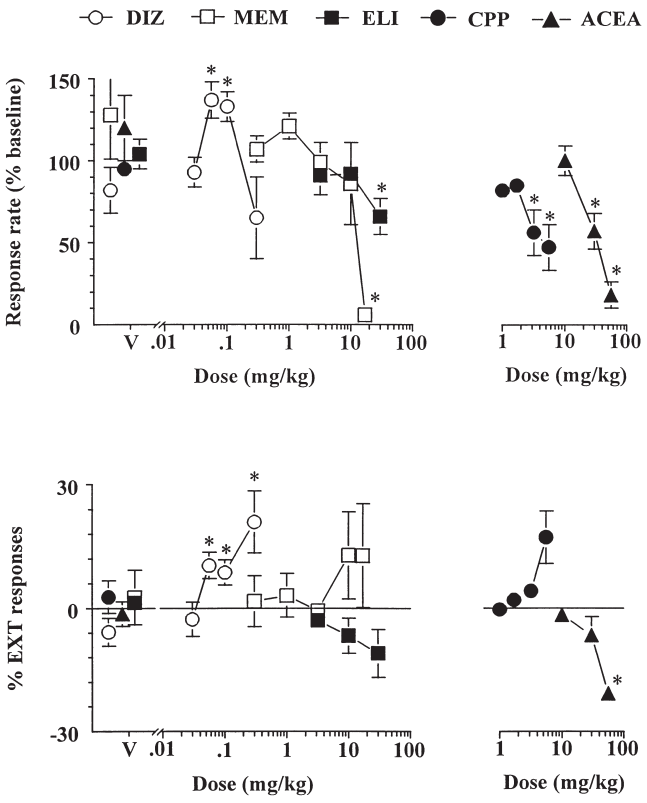

FIG. 1. Operant performance in rats pretreated with selective NMDA receptor antagonists: D-CPPen (1–5.6 mg/kg), dizocilpine (0.03–0.3 mg/kg), memantine (0.3–17 mg/kg), ACEA-1021 (10–56 mg/kg), and eliprodil (1–30 mg/kg). Upper panel, mean (±SEM) response rates (% from baseline). Lower panel, mean (±SEM) percentage of responding during the EXT component (shift from baseline). *$p < 0.05$ (Duncan's test), compared to vehicle-treated controls (data points above V′). For the sake of clarity, standard errors bars are not shown for all data points ($n = 6$).

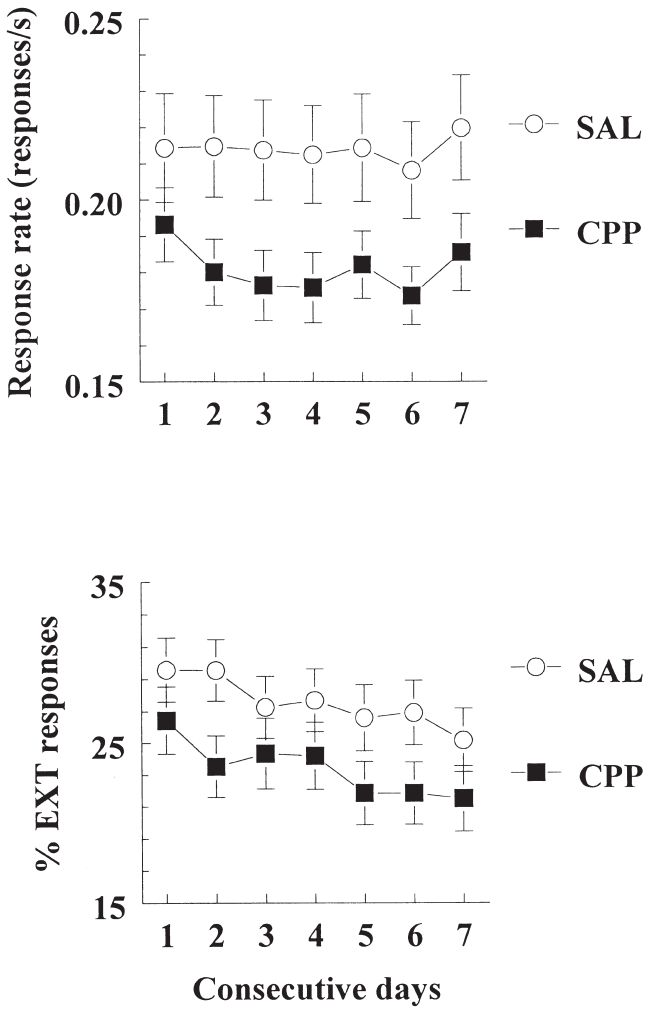

FIG. 2. Drug-free operant performance in rats during the period of repeated administration of D-CPPen (CPP) or saline (SAL). Tests were conducted 30 min prior to each of the seven repeated D-CPPen (5.6 mg/kg) or saline injections. Upper panel, mean (±SEM) response rates (% from baseline). Lower panel, mean (±SEM) percentage of responding during the EXT component ($n = 6$).

tolerance. Subsequent tests sought to evaluate effects of the following NMDA receptor antagonists in repeated D-CPPen–treated rats (tolerance transfer tests): high- and low-affinity channel blockers, dizocilpine (37) and memantine (9), respectively, as well as antagonists acting at the strychnine-insensitive glycine site [ACEA-1021, (38)] and the polyamine site [eliprodil, (1)].

METHOD

Subjects

One hundred and fifty adult experimentally naive male Wistar rats (200–250 g; Rappolovo, St. Petersburg, Russia) were kept individually in standard plastic cages with metal wire lids with free access to food and water. After a 1-week acclimation period, rats were water deprived, with the drinking bottles being introduced daily to their cages for 5 min, 4 to 5 h after the daily training session. Food (standard rodent chow from Volossovo, St. Petersburg, Russia) was available ad lib throughout the experiment. During the course of the experiments, body weight gain was on average 15 g (median: 14 g; range: 8–24 g). All experiments were conducted during the first half of the light period of a 12 L:12 D cycle (lights on at 0800 h). Experimental procedures were approved by the Ethics Committee of Pavlov Medical University, and were performed in accordance with the recommendations and policies of the U.S. National Institutes of Health "Principles of Laboratory Animal Care" (NIH publication No. 85-23, revised 1985).

Apparatus

Two identical standard operant conditioning chambers (Coulbourn Instruments, Lehigh Valley, PA) were used in the experiments. Each chamber was housed in an individual sound- and light-attenuated enclosure, ventilated with an exhaust fan. The chambers were equipped with a liquid dispenser which delivered 0.1 ml of water to a retractable drinking cup located in the center of one wall, 1.5 cm above the grid floor. A 2.8-W house light was centered at the top of the same wall. A 4.8-W white light (referred to below as the "dispenser light") was located in the same recession as the liquid dispenser, 5 cm above it. A single 3.5-cm manipulandum (retractable response lever) was located 5.5 cm to the left of the liquid dispenser. The house and dispenser lights were illuminated only when the water reinforcement schedule was operative. Recording of lever press responses, scheduling of reinforcement contingencies, and data collection were accomplished by an IBM 486/66 MHz microcomputer through a MED interface and controlled by MED-PC software (MED Associates, Inc., East Fairfield, VT).

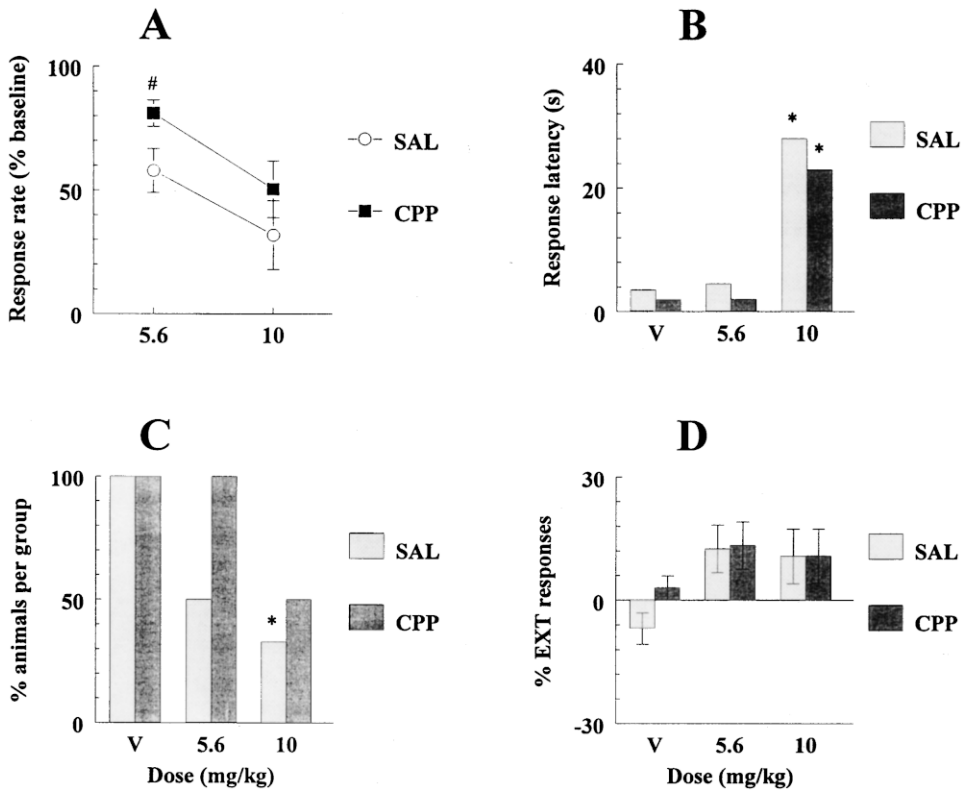

FIG. 3. Acute effects of D-CPPen (5.6 and 10 mg/kg) in rats repeatedly treated with D-CPPen (CPP, 5.6 mg/kg) or saline (SAL) once daily for 7 days. (A) Mean (±SEM) response rates (% from baseline). (B) Median group latency to the first lever press response. (C) Percentage of rats per group that earned 50 reinforcements per session. (D) Mean (±SEM) percentage of responding during the EXT component (shift from baseline). $^{*,\#}p < 0.05$ (Tukey's and Fisher's exact tests), compared to vehicle-treated controls (data points above V′) and saline-treated group (SAL), respectively ($n = 6$).

Procedure

Training and testing sessions were conducted on a daily basis Monday through Sunday. Rats were shaped to lever press for continuous water reinforcement by successive approximation technique. As soon as the animals acquired lever pressing, a multiple fixed-interval 1-s extinction 9-s (multi FI 1 s EXT 9 s) schedule of reinforcement was introduced. Events were arranged by this schedule as follows. During each EXT component, the dispenser light was illuminated for 9 s, and lever presses were recorded but had no programmed consequences. Upon termination of the EXT component, the house light was illuminated and dispenser light was extinguished, signaling the beginning of the FI component. The first lever press occurring 1 s after the FI component was started, produced a dipper delivery of water, the dispenser light was illuminated and the house light was extinguished. The dipper remained in the available position throughout the EXT component. Each training and test session began with a FI component. The experimental session lasted for 900 s or until the rat earned 50 reinforcements, whichever came first. Performance during the training sessions was judged stable when response rates for 3 consecutive days varied less than 10%. Stable responding was also characterized by the typical duration of the training sessions of less than 600 s.

Acute Administration Tests

Tests were conducted in a group of 18 rats for the following drugs: D-CPPen (1–5.6 mg/kg, 60 min presession injection time), dizocilpine (0.03–0.3 mg/kg, 15 min presession injection time), memantine (0.3–17 mg/kg, 30 min presession injection time), ACEA-1021 (10–56 mg/kg, 30 min presession injection time), and eliprodil (1–30 mg/kg, 30 min presession injection time). Drug doses and presession injection times were selected based on the preliminary experiments. Each drug dose was tested in six rats. Each subject was tested at all dose levels of no more than two NMDA antagonists. The order of tests was derived from the Latin square design. There were at least 3 days between consecutive tests. Tests were conducted provided that the following criteria were met: 1) during the three most recent training sessions maximal number of reinforcers (50) was earned, and 2) these sessions lasted less than 600 s.

Repeated Administration Tests

Preliminary studies have indicated treatment duration and doses that upon repeated administration result in significant tolerance to the operant performance-impairing effects of D-CPPen. D-CPPen (5.6 mg/kg) or saline was administered 60

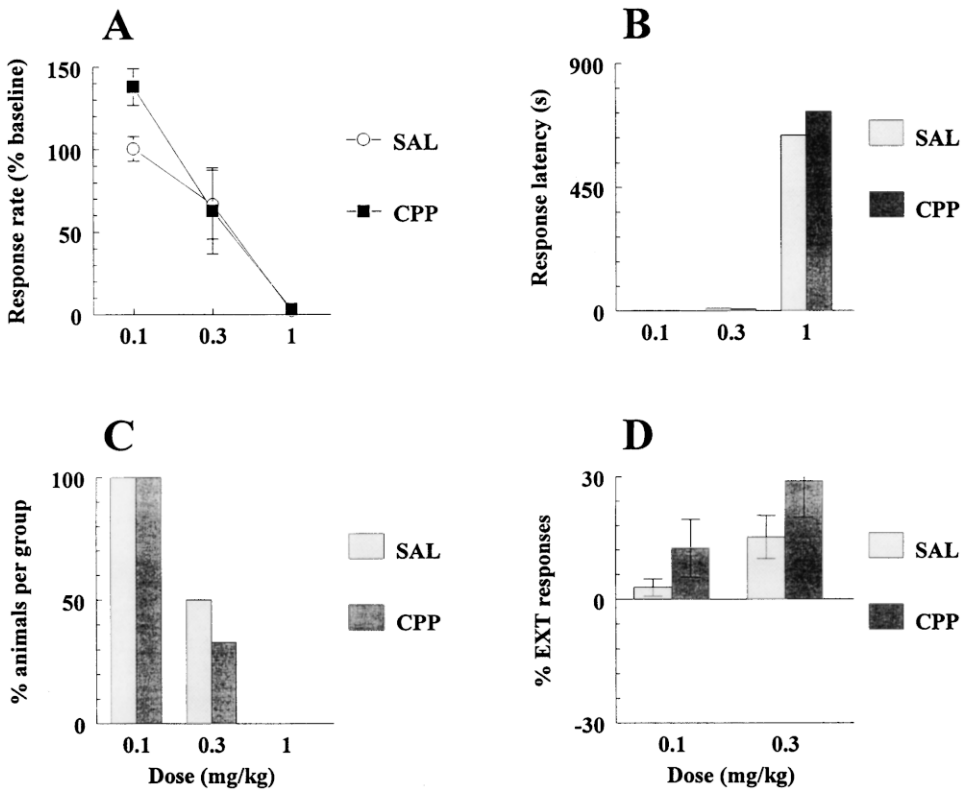

FIG. 4. Acute effects of dizocilpine (0.1, 0.3, and 1 mg/kg) in rats repeatedly treated with D-CPPen (CPP, 5.6 mg/kg) or saline (SAL) once daily for 7 days. (A) Mean (±SEM) response rates (% from baseline). (B) Median group latency to the first lever press response. (C) Percentage of rats per group that earned 50 reinforcements per session. (D) Mean (±SEM) percentage of responding during the EXT component (shift from baseline) (n = 6).

min after each experimental session for 7 consecutive days. On day 8, acute tests were conducted after pretreatment with saline or selected doses of NMDA receptor antagonists: D-CPPen (5.6 and 10 mg/kg), dizocilpine (0.1, 0.3 and 1 mg/kg), memantine (10 and 17 mg/kg), ACEA-1021 (17 and 30 mg/kg), and eliprodil (30 and 56 mg/kg). Presession injection times were as indicated above.

Data Analysis

The following parameters were recorded for each experimental session for each subject: (a) total number of lever presses emitted during the session, (b) latency to the first response, (c) total number of reinforced responses per session, and (d) number of lever presses emitted during the EXT and FI components. For the purposes of statistical analysis, these data were presented as: (a) response rate (total responses/session duration), (b) the median group latency to the first lever press response, (c) the percentage of rats per group that earned maximal allowed amount of water deliveries (i.e., 50 reinforcements per session), and (d) the percentage of EXT responses (EXT responses × 100%/total lever presses). To reduce variability of the data and emphasize the within-subject design of the analysis, the individual rat response rate and EXT responding data were subsequently converted to a percentage from baseline levels (test × 100%/baseline) and a shift from baseline (i.e., baseline value subtracted from test value), respectively. For both acute and repeated administration tests, baseline values were obtained from the session preceding the test session (e.g., day 7 for the repeated administration tests). Data from rats emitting less than 0.01 responses(s) were omitted from calculations of group EXT responding, but were included in group response rate determinations. Animals that failed to emit a single lever press per session were assigned a first response latency of 900 s.

For each treatment group, separate distribution-free one- and two-way analyses of variance (ANOVA) were conducted on the response rate; EXT responding and first response latency data with repeated measures wherever appropriate (i.e., acute administration tests). The two factors were: (a) acute NMDA receptor antagonist dose, and (b) repeated saline vs. D-CPPen treatment. ANOVAs were performed using SAS-STAT software (version 6.11, SAS Institute Inc., Cary, NC). Individual comparisons were performed using post hoc Dunnett's and Tukey's tests (only when the ANOVA revealed significant effects). Quantal data (percentage of rats per group that earned the maximal number of water deliveries) were subjected to probit analysis with Fisher's exact test for post hoc comparisons. The null hypothesis was rejected at the $p < 0.05$ level.

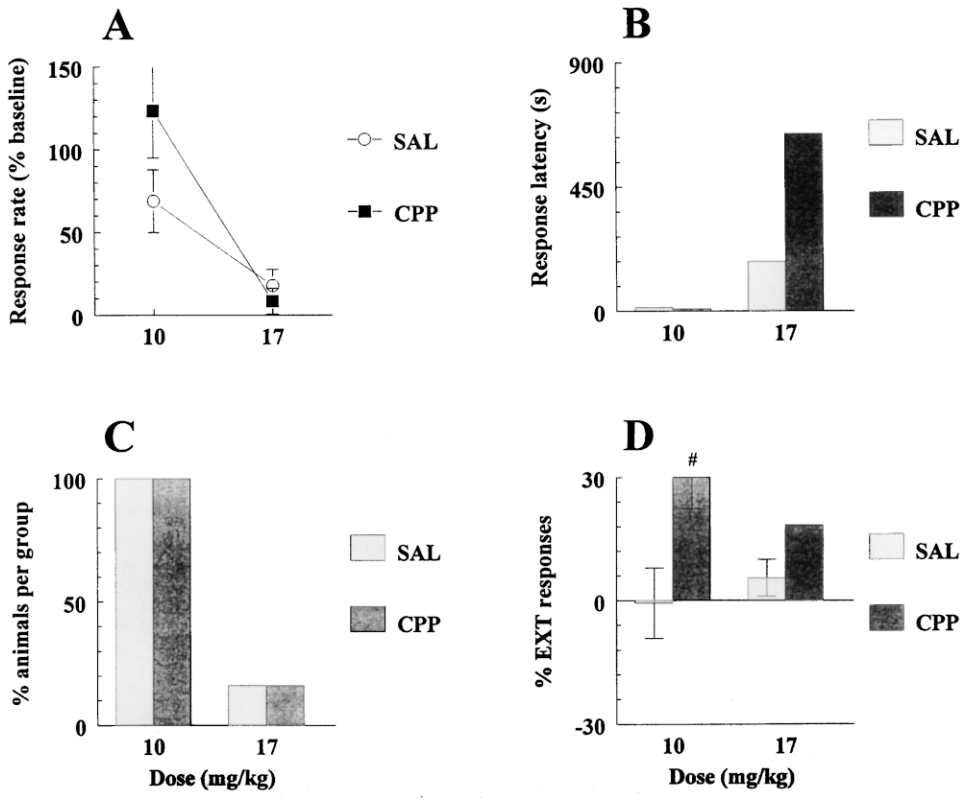

FIG. 5. Acute effects of memantine (10 and 17 mg/kg) in rats repeatedly treated with D-CPPen (CPP, 5.6 mg/kg) or saline (SAL) once daily for 7 days. (A) Mean (±SEM) response rates (% from baseline). (B) Median group latency to the first lever press response. (C) Percentage of rats per group that earned 50 reinforcements per session. (D) Mean (±SEM) percentage of responding during the EXT component (shift from baseline). $^{\#}p < 0.05$ (Tukey's test), compared to saline-treated group (SAL) (n = 6).

Drugs

The following drugs were used: D-CPPen (3-(2-carboxypiperazin-4-yl)-1-propenyl-1-phosphonic acid; SDZ EAA 494; Novartis Pharma, Basel, Switzerland), dizocilpine maleate ((+)-5-methyl-10,11-dihydro-5H-dibenzo-[a,d]-cyclohepten-5,10-imine maleate; MK-801; Research Biochemicals International, Natick, MA), memantine (1-amino-3,5-dimethyl adamantane; Merz + Co., Frankfurt am Main, Germany), ACEA-1021 (5-nitro-6,7-dichloro-1,4-dihydro-2,3-quinoxalinedione; licostinel; CoCensys, Inc., Irvine, CA), and eliprodil (Synthelabo Recherche, Bagneux, France). Dizocilpine and memantine were dissolved in physiological saline, ACEA-1021 in 50% dimethylsulfoxide, D-CPPen in equimolar sodium hydroxide in saline, and eliprodil in a vehicle of 5% Alkamuls EL-620 (castor oil ethoxylated; Rhone-Poulenc, Cranbury, NJ), and 5% ethanol (d = 0.8; actual dose—40 mg/kg). All drugs and their vehicles were injected intraperitoneally. Injection volume was 1 ml/kg. Doses are based upon the forms of the drugs listed above.

RESULTS

Acute Administration Tests

As displayed in Fig. 1, all tested antagonists affected operant performance in a dose-dependent manner. Dizocilpine at intermediate doses enhanced response rates, $F(4, 20) = 5.1$, $p < 0.01$, while higher doses of D-CPPen, memantine, ACEA-1021, and eliprodil significantly reduced rates of lever-pressing, $F(4, 20) = 5.2$, $p < 0.01$; $F(5, 25) = 4.3$, $p < 0.01$; $F(3, 15) = 9.6$, $p < 0.01$; $F(3, 15) = 3.7$, $p < 0.05$, respectively. Responding during the EXT component was significantly facilitated by dizocilpine, $F(4, 19) = 10.4$, $p < 0.01$, but reduced by ACEA-1021, $F(5, 12) = 3.8$, $p < 0.05$. D-CPPen, memantine, and eliprodil did not affect significantly EXT responding, $F(4, 19) = 1.4$, $F(5, 25) = 0.5$, $F(3, 15) = 1.4$, respectively).

Repeated Administration Tests

Across all treatment groups, the baseline response rate and EXT responding were characterized by median values of 0.18 responses/s and 32% with interquantile ranges of 0.11 responses/s and 26%, respectively. Both response rate and EXT responding remained stable during the period of repeated administration of either saline or D-CPPen (Fig. 2). Data presented in Fig. 2 were obtained in drug-free animals 23 h after the last saline/D-CPPen administration. ANOVA did not reveal any significant effect of repeated injections factor [D-CPPen by day interaction; response rate: $F(6, 889) = 0.3$; EXT responding: $F(6, 889) = 0.1$].

Repeated administration of D-CPPen was found to attenuate the ability of this drug to lower response rates (Fig. 3A) resulting in a nearly parallel upward shift of the D-CPPen dose–effect curve. Effects of both the acute and repeated D-CPPen administration were statistically significant, $F(1, 20) = 7.5$, $F(1, 20) = 5.0$, $p < 0$ .05, respectively. D-CPPen also markedly prolonged latency to the first response, $F(1, 20) = 14.7$, $p < 0$ .01, but this effect was not affected by a repeated treatment regimen (Fig. 3B). The percent of rats per group

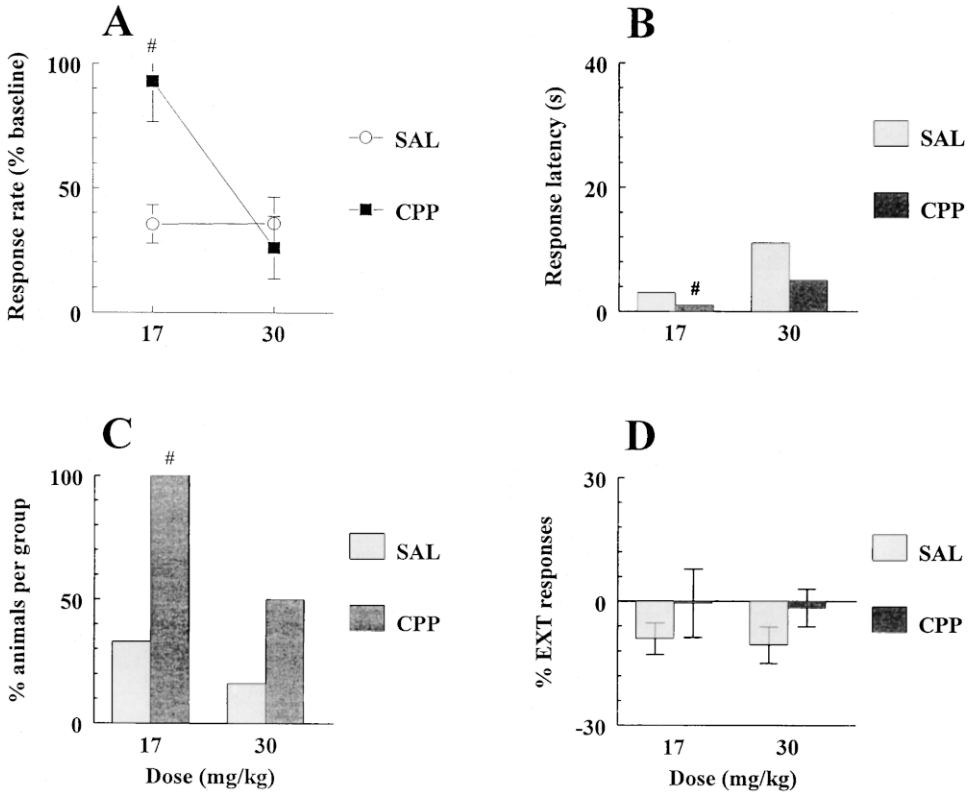

FIG. 6. Acute effects of ACEA-1021 (17 and 30 mg/kg) in rats repeatedly treated with D-CPPen (CPP, 5.6 mg/kg) or saline (SAL) once daily for 7 days. (A) Mean (±SEM) response rates (% from baseline). (B) Median group latency to the first lever press response. (C) Percentage of rats per group that earned 50 reinforcements per session. (D) Mean (±SEM) percentage of responding during the EXT component (shift from baseline). $^{\#}p < 0.05$ (Tukey's test), compared to saline-treated group (SAL) ($n = 6$).

that earned the maximum number of water deliveries per session decreased as a function of the D-CPPen dose (Fig. 3C, $\chi^2 = 7.6$, $p < 0.01$). This effect appeared to be reversed in repeated D-CPPen treated subjects ($\chi^2 = 3.1$). Similar to what was found in the acute administration tests, D-CPPen exerted a nonsignificant tendency to increase the percentage of EXT responding [Fig. 3D, $F(1, 19) = 2.2$].

Opposite to the results with acute administration of D-CPPen, the acute effects of dizocilpine seemed to be somewhat increased in rats with a history of repeated D-CPPen administration (Fig. 4). A low dose of dizocilpine (0.1 mg/kg) facilitated response rates in D-CPPen- but not in saline-treated controls (Fig. 4A). However, ANOVA did not reveal an overall effect of repeated D-CPPen treatment, $F(1, 30) = 1.1$) that precluded any between-group comparisons. Repeated D-CPPen administration did not influence the ability of dizocilpine to enhance first response latency, $F(1, 30) = 0.4$ (Fig. 4B), and to reduce the percentage of rats with maximal reinforcements (Fig. 4C; chrono $\chi^2 = 0.3$). Meanwhile, the dose of dizocilpine was a significant determinant of the lever-press rate, $F(2, 30) = 27.9$, $p < 0$ .01), first response latency, $F(2, 30) = 41.2$, $p < 0$ .01, and the proportion of rats earning 50 reinforcements per session, ($\chi^2 = 14.0$, $p < 0$ .01). EXT responding was markedly enhanced by pre-treatment with dizocilpine (Fig. 4D), $F(1, 19) = 17.6$, $p < 0$ .01. These effects were potentiated in D-CPPen–treated rats, $F(1, 19) = 5.8$, $p < 0$ .05.

Similarly, memantine dose dependently diminished the rate of lever pressing (Fig. 5A), $F(1, 20) = 20.9$, $p < 0.01$, and the percentage of rats with 50 water deliveries per session (Fig. 5C) ($\chi^2 = 9.1$, $p < 0$ .05), but chronic administration of D-CPPen did not influence any of these measures, $F(1, 20) = 1.5$, $\chi^2 = 0.4$, respectively. Latency to the first response was increased by pretreatment with memantine, and this effect was more evident in D-CPPen–treated rats [Fig. 5B; D-CPPen treatment by memantine dose interaction: $F(1, 20) = 5.4$, $p < 0$ .05]. In the D-CPPen–treated group, following an injection of 17 mg/kg of memantine, only one out of six rats was able to emit more than 10 responses per session and was included for EXT responding analysis. Nevertheless, statistical analysis found that repeated exposures to D-CPPen significantly potentiated the ability of memantine (10 mg/kg) to the enhance percentage of EXT component responses (Fig. 5D), $F(1, 10) = 6.9$, $p < 0$ .05.

Effects of ACEA-1021 were also altered by pre-exposure to D-CPPen (Fig. 6). In D-CPPen–treated rats, ACEA-1021 produced smaller decreases in response rates [Fig. 6A; D-CPPen treatment by ACEA-1021 dose interaction, $F(1, 19) = 7.1$, $p < 0.05$], first response latencies were shorter (Fig. 6B), $F(1, 20) = 4.6$, $p < 0.05$, and more rats earned 50 reinforcements per session (Fig. 6C), $\chi^2 = 5.2$, $p < 0$ .05. An ACEA-1021-induced decrease in EXT responding also seemed reduced in D-CPPen–experienced rats, and was indistinguishable from the levels observed in saline-treated rats (Fig. 6D), $F(1, 14) = 1.6$.

The decrement in operant performance produced by eliprodil tended to be enhanced in D-CPPen–treated groups [Fig. 7;

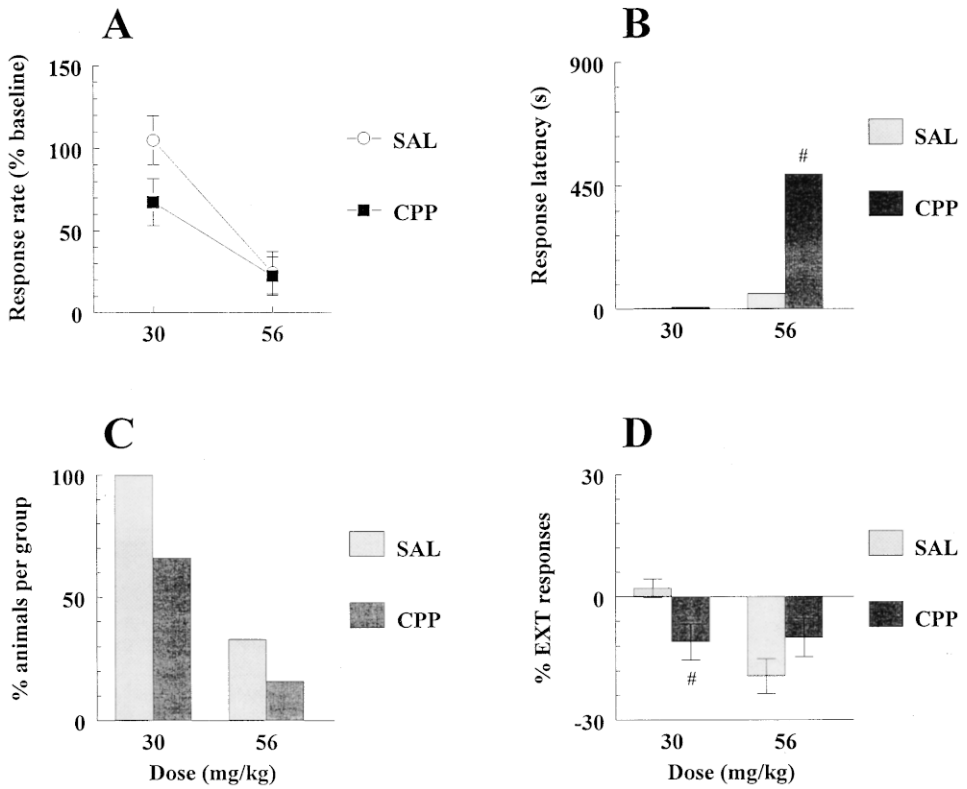

FIG. 7. Acute effects of eliprodil (30 and 56 mg/kg) in rats repeatedly treated with D-CPPen (CPP, 5.6 mg/kg) or saline (SAL) once daily for 7 days. (A) Mean (±SEM) response rates (% from baseline). (B) Median group latency to the first lever press response. (C) Percentage of rats per group that earned 50 reinforcements per session. (D) Mean (±SEM) percentage of responding during the EXT component (shift from baseline). #$p < 0.05$ (Tukey's test), compared to saline-treated group (SAL) ($n = 6$).

response rate: $F(1, 20) = 3.3$, NS, first response latency, $F(1, 20) = 5.9$, $p < 0.05$, % rats with maximal reinforcement: $\chi^2 = 2.0$, NS. Eliprodil was also found to inhibit lever pressing during the EXT component, and at the 30 mg/kg dose level this effect was significantly increased by D-CPPen preexposures (Fig. 7D; D-CPPen treatment by eliprodil dose interaction, $F(1, 16) = 6.6$, $p < 0.05$].

DISCUSSION

Operant performance was differentially affected by acute administration of the tested NMDA receptor antagonists. Dizocilpine (except for the highest dose) increased response rates, while all other tested compounds produced dose-dependent decreases in the rates of lever pressing. The effects of dizocilpine were mainly due to facilitated responding during the EXT component. This conclusion is indirectly supported by the dizocilpine dose-dependent decrements in the percentage of animals earning 50 reinforcements per session and increases in the latencies of the first response (data are not shown). These observations are in accord with the well-established ability of PCP and PCP-like NMDA receptor channel blockers to disrupt accuracy of responding (7,11). D-CPPen and memantine exhibited nonsignificant tendencies to increase EXT responding, while ACEA-1021 and eliprodil did the opposite. Earlier studies have strongly suggested remarkable differences between behavioral profiles of site-selective antagonists (2), and this evidence also extends to attention- and accuracy-impairing properties (7,18,27,35). However, one should note that within the present study acute response rate effects of dizocilpine were retested during the tolerance transfer tests, and increases in lever pressing were observed following administration of dizocilpine in repeated D-CPPen but not saline-treated rats. Thus, it appears that the acute effects of dizocilpine in the first part of this study were significantly affected by the study design that involved administration of different doses and drugs to the same animals. Although such repeated testing procedures are commonly used, our data suggest they may have limited utility for the evaluation of acute dose–effect relationships.

Response rate effects of tested compounds are apparently specific to operant performance. Using a similar study design, we tested the effects of these drug on a simple measure of locomotor activity (Bespalov, Dravolina, Zvartau, Balster, Beardsley, submitted). The response rate decreasing effects of D-CPPen and ACEA-1021 occurred at dose levels lower than those required for suppression of locomotor activity. In contrast, dizocilpine and memantine exerted stimulatory effects at doses up to 0.3 and 30 mg/kg, respectively (higher doses were not tested).

Essential differences between the tested drugs were revealed in experiments with repeated preexposures to D-CPPen. A 7-day administration of D-CPPen had little or no effect on performance during the daily training sessions. Meanwhile, D-CPPen–treated subjects were less sensitive to the acute rate-decreasing effects of D-CPPen. As indicated above in the introduction section, the evidence on tolerance to the effects of competitive NMDA receptor antagonists is quite equivocal. Although the outcome of the tolerance studies does not seem to depend on the antagonist used, the dependent variable measured may be the crucial factor determining whether or not tolerance may be established.

Although previous studies on the development of tolerance to competitive antagonists have not focused on decrement in operant performance as a dependent variable, potentially relevant information may be derived from the drug discrimination experiments where rate-decreasing effects were assessed together with substitution gradients, and the experimental design involved repeated exposures to the training drug. Careful examination of several studies that reported data on the competitive NMDA receptor antagonist, (±)-CPP, suggests that response rate-reducing effects of this compound were more evident in NMDA- (31), pentobarbital- (30), and PCP-discriminating rats (29), compared to rats discriminating another competitive antagonist, NPC 12626, from saline (32). Similarly, injection of 3 mg/kg of D-CPPen did not diminish lever pressing activity in NPC 12626-discriminating rats (5), but reduced the response rate by about 40% in a morphine discrimination study (Bespalov, Beardsley, and Balster, unpublished) and in the acute administration tests of the present study. Interestingly, in rats trained to discriminate PCP from saline, the response rate-decreasing effects of (±)-CPP seemed to increase with the decrease in the training dose [Fig. 3 in (19)]. However, one should note that for PCP itself ED_{50}s for the response rate effects remained relatively constant with decreases in the training dose (3).

The effects of repeated administration of D-CPPen depended upon both the type of antagonist used in the tolerance transfer tests and the criterion response analyzed. For response rate data, crosstolerance was seen with ACEA-1021, and was expressed as a substantial reduction of response rate decreasing effects. Earlier studies have also shown little or no crosstolerance observed between glycine site partial agonists and competitive NMDA receptor antagonists (21,23). Meanwhile, repeated exposures to D-CPPen differently affected acute drug-induced alterations of EXT responding. ACEA-1021 was the only tested compound that lowered percentage of responses emitted during the EXT component in D-CPPen–naive rats. This effect was reversed in D-CPPen–treated subjects. Overall, acute effects of ACEA-1021 seemed to be affected by D-CPPen administration more than any other compound. All four recorded behavioral measures consistently showed significant tolerance development.

Contrary to the ACEA-1021 test data, the response rate effects of dizocipine seemed to be potentiated in D-CPPen–treated subjects. These data are in apparent contrast with previous demonstrations of crosstolerance between PCP-like channel blockers and competitive antagonists [(17); see, however, (22)]. There is also evidence for the development of tolerance to dizocilpine's decremental effects on operant responding (28). The ability of dizocilpine to enhance lever-press activity during the EXT component was also significantly increased. Similarly, memantine, which did not have significant effects on EXT responding in D-CPPen–naive rats, markedly increased this index in D-CPPen–treated subjects. One plausible explanation of these results might be that D-CPPen–treated rats developed some tolerance to the rate-decreasing effects of dizocilpine, allowing the stimulant effects to be more powerfully revealed.

Sensitization rather than tolerance was also observed for behavioral effects of eliprodil following repeated administration of D-CPPen. However, unlike dizocilpine, in D-CPPen–treated rats eliprodil decreased EXT responding while exerting suppressive effects on operant performance parameters (response rate, latency to the first response, proportion of rats with 50 reinforcements per session).

Based on the present results, one can suggest that repeated administration of competitive NMDA receptor antagonist differentially affects the expression of various sites on the NMDA receptor complex. Although reciprocal relationships between various recognition sites have long been suspected

[e.g., glutamate and glycine sites, (25)], the available experimental evidence cannot support any further speculations because overall information is quite controversial and very limited with regard to the repeated antagonist administration-induced changes in glycine and polyamine sites. For example, repeated administration of CGP39551 or CGP37849 produced tolerance to their sedative effects but did not affect dizocilpine binding in cerebrocortical tissue (21). Nevertheless, the density of dizocilpine-binding sites on cultured cortical neurons was found to be up-regulated significantly by in vitro exposure to CGS 19755 or d-AP5 (34).

In conclusion, acute administration of NMDA receptor antagonists differentially affected operant responding. Both increases and decreases in response rates and accuracy of responding were observed. Repeated preexposure to D-CPPen attenuated behavioral disruption due to D-CPPen or ACEA-1021 administration but potentiated the effects of dizocilpine, memantine and eliprodil.

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