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Stress and a Glycinergic Intervention Interact in the Modulation of MK-801–Elicited Mouse Popping Behavior

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DEUTSCH, S. I., R. B. ROSSE, B. L. SCHWARTZ, D. G. POWELL AND J. MASTROPAOLO. *Stress and a glycinergic intervention interact in the modulation of MK-801–elicited mouse popping behavior.* PHARMACOL BIOCHEM BEHAV **62**(2) 395–398, 1999.—The ability of D-cycloserine, a partial glycine agonist, to modulate mouse popping behavior elicited by MK-801, a noncompetitive NMDA receptor antagonist, was studied in unstressed and stressed mice. In unstressed animals, D-cycloserine (5.6 and 10 mg/kg) attenuated the ability of MK-801 (1.0 mg/kg) to elicit this behavior. However, the ability of D-cycloserine to attenuate MK-801–elicited mouse-popping behavior was not evident in stressed mice, 24 h after they were forced to swim for up to 10 min in cold water. Thus, the therapeutic value of glycinergic interventions may be limited by environmental factors, such as stress. © 1999 Elsevier Science Inc.

NMDA receptor MK-801 Popping behavior D-Cycloserine Phencyclidine

RODENT behaviors elicited by noncompetitive antagonists of the *N*-methyl-D-aspartate acid (NMDA) receptor complex, such as phencyclidine (PCP) and MK-801 (dizolcipine), may be useful in the screening of compounds with unique antipsychotic properties (3,4). In these preclinical screening procedures, a candidate compound is identified by its ability to attenuate PCP/MK-801–elicited rodent behaviors; the procedures are largely derived from the clinical observation that PCP precipitates a schizophreniform psychosis in susceptible individuals (6,10). PCP and MK-801 are classified as "open-channel" blockers because their actions result from binding to a hydrophobic domain within the NMDA-associated ionophore; access to this domain requires the channel to be in its open or "active" configuration.

Glycine, an NMDA agonist, was reported to antagonize PCP-induced hyperactivity in mice (14). The partial agonist (+)-HA-966 has also been reported to inhibit MK-801–induced hyperactivity (1). Interestingly, D-cycloserine, a partial glycine agonist at the strychnine-insensitive glycine-binding site, has been shown to increase MK-801–induced hyperactivity (1). These findings have led to speculations that the NMDA agonist glycine and (+)-HA-966 might be better candidate antipsychotic agents than D-cycloserine (1).

An alternative conceptualization is that exacerbation of PCP/MK-801–elicited behaviors by a candidate antipsychotic compound reflects its facilitation of NMDA receptor-mediated neurotransmission and activation of the channel. This augmented NMDA receptor-mediated neurotransmission is associated with greater accessibility of noncompetitive NMDA receptor antagonists to their site of action within the channel (9).

MK-801 elicits episodes of intense jumping behavior in mice, referred to as popping (4,7–9,12). The elicitation of this behavior might reflect a unique interaction of MK-801 with its specific channel domain, rather than simple antagonism of NMDA receptor-mediated neurotransmission. Evidence that suggests a unique interaction is that popping could not be elicited by 3-(2-carboxypiperazine-4-yl)propyl-1-phosphate (CPP), a competitive antagonist, over a dosage range of 3.2 to 32 mg/kg (9). In this study (9), treating mice with CPP (10 mg/kg) prior to the administration of MK-801 (0.56 to 1.8 mg/kg) sig-

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nificantly attenuated the ability of MK-801 to elicit popping behavior. Conceivably, this attenuation of MK-801–elicited popping behavior by pretreatment with CPP results from fewer channels in the "active" or open configuration. If popping behavior was due simply to interference with NMDA receptor-mediated neurotransmission, then the interaction of CPP and MK-801 on popping behavior would have been either additive or synergistic. Further evidence of a unique interaction between MK-801 and the NMDA receptor channel was that MK-801 could substitute for ketamine in rhesus monkeys trained to discriminate between ketamine and saline. In contrast, CGS 19755, a competitive NMDA receptor antagonist, could not substitute for ketamine (15). Thus, behavioral procedures may be useful in discriminating functional differences in the properties of competitive and noncompetitive NMDA receptor antagonists (2,13,15).

The current investigation examined the ability of D-cycloserine at different doses to influence MK-801–elicited popping behavior in control mice and mice forced to swim for up to 10 min in cold water.

METHOD

Animals

Outbred NIH Swiss mice weighing 20 to 30 g were obtained from the National Cancer Institute (Frederick, MD), housed in hanging clear Plexiglas cages in groups of 12, and maintained on a 12 L:12 D cycle with free access to food and water. The mice were transported to the laboratory on the day of the experiment. Animals were weighed individually prior to both drug injection and automated assessment of motor behaviors. Group sizes were not equal due to attrition from the stress procedures and multiple injections; they ranged from 6 to 12 animals.

Stress Procedure

Mice were forced to swim in cold (6°C) water for up to 10 min, 24 h prior to testing the ability of MK-801 to elicit popping behavior.

Drugs

MK-801 (Dizolcipine) was purchased from Research Biochemicals Inc. (Natick, MA). D-Cycloserine was purchased from the Aldrich Chemical Co. (Milwaukee, WI). MK-801 and D-cycloserine were dissolved in deionized water and prepared as needed on the day of each experiment. They were injected intraperitoneally in a volume of 0.01 ml/g of body weight. D-Cycloserine (5.6, 10, or 18 mg/kg) or its vehicle was administered 30 min before the MK-801 injection; MK-801 (0.56, 1 or 1.8 mg/kg) or its vehicle was injected 1 min prior to the 30-min monitoring period in both stressed and unstressed animals. Computerized monitoring of mouse popping behavior is described below.

Computerized Assessment of MK-801–Elicited Popping

The recording of MK-801–induced popping behavior was divided into two phases: a baseline period of 5 min (which followed the administration of a pretreatment of D-cycloserine or its vehicle 25 min earlier), and an outcome recording period of 30 min, which immediately followed an injection of either MK-801 or its vehicle.

The automated system for measuring MK-801–elicited mouse popping is based on the detection and measurement of vertical displacements of a platform related to mouse movements (12). The vertical displacements resulting from mouse "pops" are detected and converted to electrical signals (S72-25 Type A Transducer Coupler and S75-01 Modified Contour

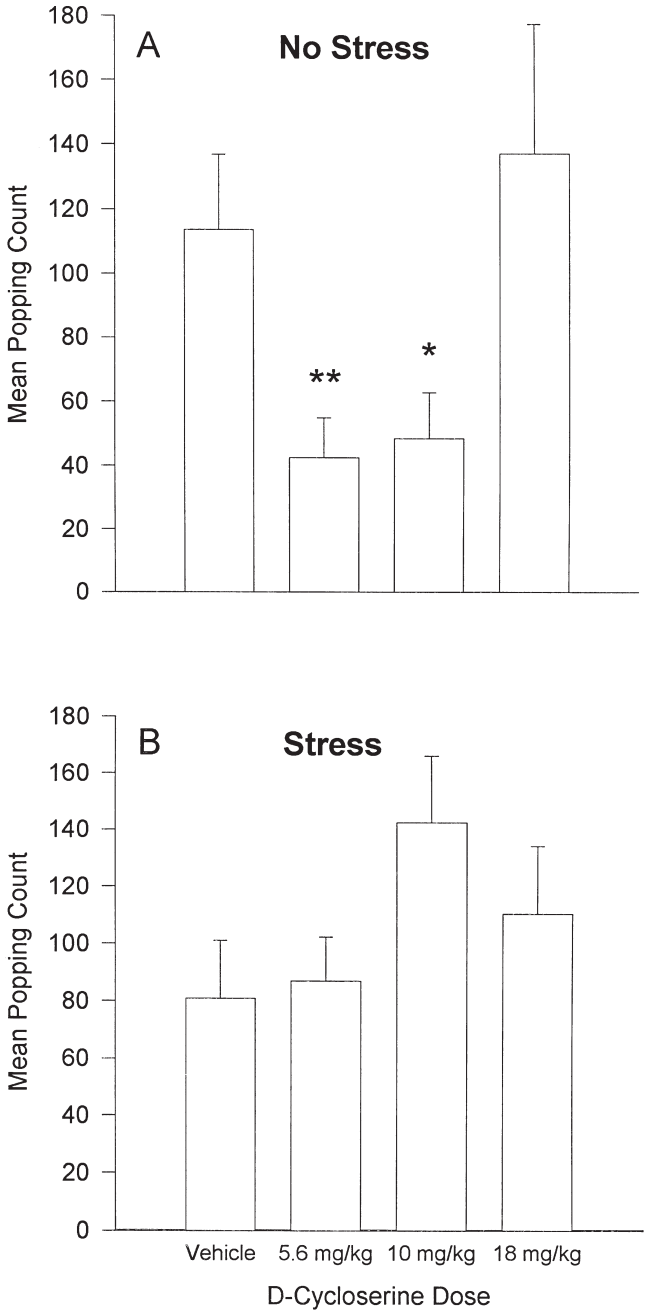

FIG. 1. (A) Mean (± SEM) number of pops for groups of unstressed mice injected (IP) with either vehicle or D-cycloserine (5.6, 10.0, and 18.0 mg/kg) 30 min prior to an injection of MK-801 (1.0 mg/kg). The *p*-value for the 5.6 mg/kg dose of D-cycloserine was 0.014, and the *p*-value for the 10.0 mg/kg dose was 0.027, compared to the vehicle condition, respectively. (B) Mean (± SEM) number of pops for groups of stressed mice injected (IP) with either vehicle or D-cycloserine (5.6, 10.0, and 18.0 mg/kg) 30 min prior to an injection of MK-801 (1.0 mg/kg).

TABLE 1
POPPING DATA FOR ALL STRESS BY D-CYCLOSERINE DOSE BY MK-801 DOSE CONDITIONS

		D-Cycloserine Dose			
	MK-801 Dose	Vehicle	5.6	10	18
No stress	Vehicle	3.5 (1.2) {8}	4.78 (1.69) {9}	1.6 (0.62) {10}	12.00 (4.37) {11}
Stress	Vehicle	0.375 (0.26) {8}	4.00 (1.62) {7}	0.88 (0.58) {8}	1.00 (0.42) {10}
No stress	0.56 mg/kg	68.38 (20.12) {8}	81.44 (27.49) {9}	50.67 (14.75) {9}	10.00 (2.16) {11}
Stress	0.56 mg/kg	24.6 (7.99) {10}	22.36 (8.37) {11}	94.00 (21.65) {11}	56.91 (16.20) {11}
No stress	1.0 mg/kg	113.55 (23.29) {11}	42.46 (12.34) {11}	48.45 (14.19) {11}	137.10 (40.32) {10}
Stress	1.0 mg/kg	80.82 (20.17) {11}	86.92 (15.20) {12}	142.30 (23.75) {10}	110.27 (23.78) {11}
No stress	1.8 mg/kg	93.75 (18.26) {12}	89.25 (15.38) {12}	79.46 (22.97) {11}	79.67 (22.36) {6}
Stress	1.8 mg/kg	102.88 (39.36) {8}	64.91 (20.06) {11}	76.46 (14.91) {11}	111.25 (21.66) {12}

Each cell shows the mean popping count, standard error ( ), and n { } for each group.

Following Integrator; Coulborn Instruments, Allentown, PA), and are then transformed into a digital signal (L25-12 A/D Converter; Coulborn Instruments). The chamber, which housed the animal for the experimental session, measured 16.5 cm long, 8.9 cm wide, and 8.9 cm high. A discrete count of popping is defined as a vertical displacement of the platform of more than 150% of body weight. The computer is able to determine the total number of popping counts, force (in gram equivalents) of individual pops, and the duration of an episode of popping (in seconds). Reverberations or "aftershock" movements of the platform after jumps are removed automatically by the system in the manner used in the measurement of startle responses in laboratory animals (Coulborn Instruments, Inc., Allentown, PA).

RESULTS

The data were subjected to a three-way analysis of variance (ANOVA) in a 2 (stress/unstress) × 4 (D-cycloserine doses) × 4 (MK-801 doses) design. The results of the overall ANOVA revealed a significant main effect for MK-801, $F(3, 31) = 38.31$, $p < 0.001$. Although there were no other significant main effects, there was a significant two-way, D-cycloserine by stress interaction, $F(3, 31) = 2.97$, $p = 0.032$, and a significant three-way, stress by D-cycloserine by MK-801 interaction, $F(9, 31) = 2.65$, $p = 0.006$. To clarify the three-way interaction and examine the effects of stress on D-cycloserine at each dose of MK-801, further analyses were performed. Separate one-way ANOVAs revealed that the 5.6 mg/kg, $F(1, 21) = 7.27$, $p = 0.014$, and 10.0 mg/kg, $F(1, 21) = 5.69$, $p = 0.027$, doses of D-cycloserine significantly reduced the number of pops in unstressed mice at the 1.0 mg/kg dose of MK-801. However, these doses of D-cycloserine did not significantly change the mean number of pops in stressed mice (see Fig. 1). The mean number of pops (standard error and *n* per group) in the 30-min recording period is presented for all 32 treatment conditions in Table 1.

DISCUSSION

In prior reports, we showed that popping behavior varies as a function of the dose of MK-801 (4,7–9,12). The results reported here revealed that D-cycloserine affected this pattern. Specifically, the 5.6 and 10.0 mg/kg doses of D-cycloserine decreased the amount of popping behavior that was observed with 1.0 mg/kg of MK-801. Also, the data showed a significant interaction between stress and D-cycloserine. The "stress-associated" alteration of the ability of D-cycloserine to modulate a behavioral effect of MK-801 is consistent with the results of an independent biochemical study. In this study, glycine's potency to competitively inhibit the binding of 5, 7-^{3}H-dichlorokynurenic acid (5,7-^{3}H-DCKA) to the strychnine-insensitive site differed in frontocortical tissue obtained from unstressed and stressed rats (11). Thus, stress was associated with a measurable change in the ability of glycine to bind to the NMDA receptor complex. Our results suggest that the biochemical change in the strychnine-insensitive binding site associated with stress is behaviorally relevant.

Pathologic alteration of NMDA receptor-mediated neurotransmission has been proposed to occur in a variety of neuropsychiatric disorders (5). Animal paradigms for the assessment of a compound's ability to facilitate or dampen NMDA receptor-mediated neurotransmission should facilitate the process of medication development for several of these disorders. The current data suggest that the therapeutic value of glycinergic interventions for the treatment of schizophrenia may be limited by acute environmental stress, and the dosage range of D-cycloserine for this indication may be narrow. The computerized assessment of MK-801–elicited mouse popping behavior may serve as a useful "intact animal" procedure for the assessment of a compound's ability to influence NMDA receptor-mediated neurotransmission.

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REFERENCES

1. Carlsson, M. L.; Engberg, G.; Carlsson, A.: Effects of D-cycloserine and (+)-HA-966 on the locomotor stimulation induced by NMDA antagonists and clonidine in monoamine-depleted mice. J. Neural Transm. [Gen. Sect.] 95:223–233; 1994.
2. Cole, B. J.; Klewer, M.; Jones, G. H.; Stephens, D. N.: Contrasting effects of the competitive NMDA antagonist CPP and the noncompetitive NMDA antagonist MK-801 on performance of an operant delayed matching to position task in rats. Psychopharmacology (Berlin) 111:465–471; 1993.
3. Corbett, R.; Camacho, F.; Woods, A. T.; Kerman, L. L.; Fishkin,

R. J.; Brooks, K.; Dunn, R. W.: Antipsychotic agents antagonize non-competitive *N*-methyl-D-aspartate antagonist-induced behaviors. Psychopharmacology (Berlin) 120:67–74; 1995.
4. Deutsch, S. I.; Hitri, A.: Measurement of an explosive behavior in the mouse, induced by MK-801, a PCP analogue. Clin. Neuropharmacol. 16:251–257; 1993.
5. Deutsch, S. I.; Mastropaolo, J.; Rosse, R. B.: Behavioral approaches to the functional assessment of NMDA-mediated neural transmission in intact mice. Clin. Neuropharmacol. 20: 375–384; 1997.
6. Deutsch, S. I.; Mastropaolo, J.; Schwartz, B. L.; Rosse, R. B.; Morihisa, J. M.: A "glutamatergic hypothesis" of schizophrenia. Rationale for pharmacotherapy with glycine. Clin. Neuropharmacol. 12:1–13; 1989.
7. Deutsch, S. I.; Rosse, R. B.; Paul, S. M.; Riggs, R. L.; Mastropaolo, J.: Inbred mouse strains differ in sensitivity to "popping" behavior elicited by MK-801. Pharmacol. Biochem. Behav. 57:315–317; 1997.
8. Deutsch, S. I.; Rosse, R. B.; Paul, S. M.; Tomasino, V.; Koetzner, L.; Morn, C. B.; Mastropaolo, J.: 7-Nitroindazole and methylene blue, inhibitors of neuronal nitric oxide synthase and NO-stimulated guanylate cyclase, block MK-801-elicited behaviors in mice. Neuropsychopharmacology 15:37–43; 1996.
9. Deutsch, S. I.; Rosse, R. B.; Riggs, R. L.; Koetzner, L.; Mastropaolo, J.: The competitive NMDA antagonist CPP blocks MK-801-elicited popping behavior in mice. Neuropsychopharmacology 15: 329–331; 1996.
10. Javitt, D. C.; Zukin, S. R.: Recent advances in the phencyclidine model of schizophrenia. Am. J. Psychiatry 148:1301–1308; 1991.
11. Nowak, G.; Redmond, A.; McNamara, M.; Paul, I. A.: Swim stress increases the potency of glycine at the *N*-methyl-D-aspartate receptor complex. J. Neurochem. 64:925–927; 1995.
12. Rosse, R. B.; Mastropaolo, J.; Koetzner, L.; Morn, C. B.; Sussman, D. M.; Deutsch, S. I.: Computerized measurement of MK-801 elicited hyperactivity and popping in mice. Clin. Neuropharmacol. 18:448–457; 1995.
13. Svensson, A.; Pileblad, E.; Carlsson, A.: A comparison between the non-competitive NMDA antagonist dizocilipine (MK-801) and the competitive NMDA antagonist D-CCPene with regard to dopamine turnover and locomotor-stimulatory properties in mice. J. Neural Transm. [Gen. Sect.] 85:117–129; 1991.
14. Toth, E.; Lajtha, A.: Antagonism of phencyclidine-induced hyperactivity by glycine in mice. Neurochem. Res. 11:393–400; 1986.
15. Winger, G.; France, C. P.; Wood, J. H.: Comparison of CGS 19755 and phencyclidine-like compounds. In: Meldrum, B. S.; Moroni, F.; Simon, R. P.; Woods, J. H., eds. Excitatory amino acids. New York: Raven Press; 1991:539–545.