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A Further Study on Asymmetric Cross-Sensitization Between MK-801 and Phencyclidine-Induced Ambulatory Activity

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XU, X. AND E. F. DOMINO. *A further study on asymmetric cross-sensitization between MK-801 and phencyclidine-induced ambulatory activity.* PHARMACOL BIOCHEM BEHAV **63**(3) 413–416, 1999.—Our previous study found that MK-801–sensitized rats showed cross-sensitization to the locomotor stimulant effects of phencyclidine, but phencyclidine sensitized rats did not show cross-sensitizaton to MK-801. This study was designed to determine whether the asymmetric cross-sensitization was due to injection–environment conditioning or possibly reduced phencyclidine-like effects following further repeated injections of phencyclidine. Adult female Sprague–Dawley rats were used in this study, and their activity was assessed with an automated photoelectric system. Results confirmed the early finding that four daily injections of phencyclidine (3.2 mg/kg) or MK-801 (0.32 mg/kg) produced locomotor sensitization, and that the two drugs showed asymmetric cross-sensitization. Moreover, injection–environment conditioning was ruled out as a possible cause for cross-sensitization from MK-801 to phencyclidine, and possibly reduced phencyclidine-like effects following further repeated injections was also ruled out as a cause for the failure of cross-sensitization from phencyclidine to MK-801. These additional results further confirm our previous finding, and indicate that there are significant differences in the neural mechanisms underlying phencyclidine- and MK-801–induced sensitization. © 1999 Elsevier Science Inc.

Phencyclidine (PCP) Dizocilpine (MK-801) Reverse tolerance Behavioral sensitization Locomotor activity Ambulation N-allylnormetazocine (NANM, SKF-10,047)

PHENCYCLIDINE exerts its pharmacological effects through several neurotransmitter systems (9). It has been believed that the primary action of phencyclidine is to selectively reduce the excitatory efects of glutamic acid through blockade of the ion channel of *N*-methyl-D-aspartate (NMDA) receptors (1,4). However, phencyclidine is also known to enhance dopamine (DA) transmission (12), and has anticholinergic (5,13), serotonergic (7,14), and sigma actions. About 2 decades ago, Martin, Eades, Thompson, Huppler, and Gilbert (11) proposed that sigma receptors mediate certain opioid effects based upon the actions of racemic SKF-10,047 (N-allylnormetazocine, NANM). It is now clear that (+)NANM in vivo has phencyclidine-like but not opioid-like actions. The acute and chronic locomotor stimulant effects of phencyclidine and (+)NANM, a proposed sigma ligand, are considerably similar, with both showing reciprocal cross-sensitization (6,8). Sigma receptor-mediated effects are different than those mediated by either opioid receptors or the PCP binding site on NMDA receptors (3,10,15).

As the primary action of phencyclidine is to reduce the excitatory activity of NMDA receptors, it was expected and was also found that MK-801 (a selective noncompetitive NMDA antagonist) sensitized rats show cross-sensitization to the locomotor stimulant effects of phencyclidine. Unexpectedly, phencyclidine-sensitized rats did not show cross-sensitization to MK-801 (19). The failure of cross-sensitization from phencyclidine to MK-801 may result from a decrease in locomotor activity following further repeated phencyclidine injections. It

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has been shown that sensitization to phencyclidine-induced locomotor activity in rodents develops after four daily systemic injections. However, the increase in locomotor activity is followed by a decrease to the day 1 level of locomotor activity over 14 consecutive daily injections (2). Furthermore, cross-sensitization from MK-801 to phencyclidine may be due to injection–environmental conditioning. That is, the MK-801–sensitized rats associated injection procedures in the experimental environment with increased locomotor activity. As a result, when given an injection in the experimental environment, they showed increases in locomotor activity. The present report addressed these two possibilities. The results ruled those out and support the original finding of asymmetric cross-sensitization between MK-801 and phencyclidine.

METHOD

Subjects

Adult female Sprague–Dawley rats (Harlan–Sprague–Dawley, Inc., Indianapolis, IN) weighing 200–250 g, were allowed at least 1 week of acclimatization to the animal facilities. During this time, as well as during the subsequent experimental period, the rats were housed two or three per cage with unlimited access to food and water in a rodent room with constant temperature, humidity, and a 12 L:12 D cycle (0700–1900 light). The experiments were conducted during the light phase of day–night cycle.

Apparatus

The locomotor activity of each animal was measured with the Digiscan "Micro" system consisting of four mounting frames and one analyzer (Omnitech Electronics, Columbus, OH). A mounting frame contained two parallel panels, one photocell panel with 16 infrared light beams spaced 2.54 cm apart, and one light beam detector panel. Each rat was placed in a transparent Plexiglas cage (46 × 24 × 18 cm) within a mounting frame located in an illuminated sound dampened chamber. The Digiscan system detected animal's activity by counting light beam interruptions. Ambulatory activity is defined as interruptions of two consecutive light beams caused by the animal moving from one location to another. Thus, ambulation is separate from the total activity, which also contains repetitive interruptions of the same light beams produced by stereotyped movements. Data were automatically recorded and processed by the analyzer, and further transferred to and stored on a Macintoch IIsi computer.

Drugs

Phencyclidine (National Institute on Drug Abuse, Rockville, MD) was dissolved in dilute HCl and saline (0.9% NaCl) solution. The drug was then neutralized with NaOH to give a final pH of approximately 6.4. (+)MK-801 hydrogen maleate (Research Biochemicals, Inc., Natick, MA) was dissolved in saline. Phencyclidine, in a dose of 3.2 mg/kg, and MK-801, in a dose of 0.32 mg/kg, were administered IP because the same dosages and route of drug administration were used in the previous study (19).

Procedure

Rats were randomly divided into three groups. Groups were injected with either saline, phencyclidine, or MK-801 daily for 4 consecutive days. On the fifth day, half of the daily saline-treated rats received phencyclidine and the other half received MK-801. Half of the daily phencyclidine-treated rats received MK-801 and the other half received phencyclidine. Daily MK-801–treated rats received phencyclidine. On the sixth day, daily phencyclidine-treated rats and daily MK-801–treated rats received saline. Rats were assessed every day, and their ambulatory activity was measured immediately following injection of each agent. Each animal's activity was monitored continuously for the next 120 min.

RESULTS

A two-way ANOVA with one between factor (treatment: saline/PCP/MK-801) and one within factor (injection: day 1/day 4) on ambulatory activity (Fig. 1) indicated significant effects of treatment, $F(2, 24) = 59.04$, $p < 0.001$], injection, $F(1, 24) = 20.94$, $p < 0.001$, and interaction, $F(2, 24) = 16.96$, $p < 0.001$. Further correlated *t*-tests showed that while daily saline IP injection did not significantly change ambulation, daily administration of 3.2 mg/kg IP of phencyclidine resulted in a significantly enhanced locomotor stimulant effect over 4 consecutive days ($p < 0.01$). Daily administration of 0.32 mg/kg IP of MK-801 did not further enhance its locomotor stimulation over 4 consecutive days in a couple of rats. These rats were excluded in the present study so that only MK-801–sensitized rats were used to investigate cross-sensitization. Daily MK-801 produced sensitization in the majority of rats treated with MK-801, as shown in Fig. 1. The difference in ambulatory activity following the first compared to the fourth injection of MK-801 of rats that showed a progressive increase in ambulatory activity was also statistically significant ($p < 0.01$).

On day 5, asymmetric cross-sensitization occurred between MK-801 and phencyclidine. The phencyclidine-induced ambulatory activity was significantly enhanced after four daily MK-801 injections ($p < 0.001$), whereas the MK-801–stimulated ambulatory activity did not show a significant change after four daily phencyclidine injections (Fig. 2).

Figure 3 shows that daily phencyclidine-treated rats continuously showed significant increases in ambulatory activity on day 5. A one-way ANOVA with repeated measures indicated a significant effects of injections, $F(4, 20) = 12.12$, $p < 0.001$. Multiple comparisons revealed that increases in ambulatory activity on day 4 and day 5 were significant (Fig. 3).

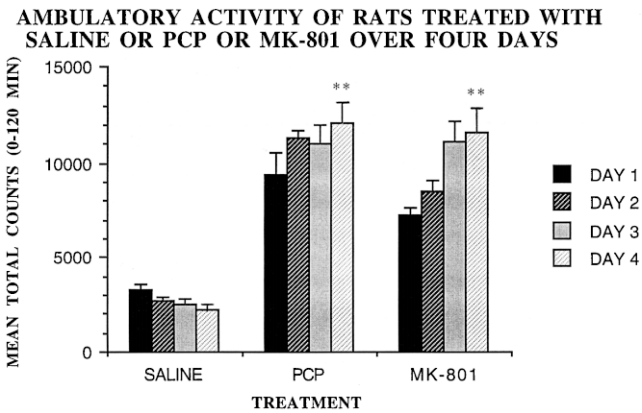

FIG. 1. Ambulatory activity of rats treated with either saline or phencyclidine (PCP) or MK-801 over 4 consecutive days. Each bar represents the mean total counts for ambulation in 120 min ± SE for 6 to 13 rats. $^{**}p < 0.01$ compared with related activity on day 1.

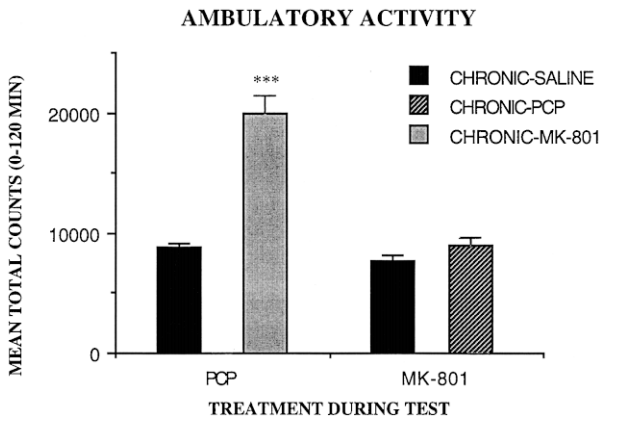

FIG. 2. Phencyclidine- and MK-801–induced ambulatory activity after chronic pretreatment with saline, phencyclidine, or MK-801, respectively. Each bar represents the mean total counts for ambulation in 120 min ± SE for six to eight rats. ***$p < 0.001$ compared with ambulatory activity of chronic-saline rats.

Figure 4 shows that neither phencyclidine- nor MK-801–sensitized rats showed any locomotor stimulant effects after saline injection on day 6. A one-way ANOVA with multiple comparisons revealed that phencyclidine- and MK-801–sensitized rats showed less ambulatory activity following a saline injection on day 6, but not significantly less than daily saline-treated rats, $F(2, 24) = 2.348, p > 0.05$. The multiple comparisons indicated that daily MK-801–treated rats showed significantly less ambulatory activity than daily saline-treated rats.

DISCUSSION

The present study confirmed the previous finding that chronic administration of phencyclidine results in an enhanced locomotor stimulant effect or sensitization (2,6,8,16). Chronic administration of MK-801 also sensitized rats to the locomotor stimulant effects of MK-801 in the present study, confirming previous reports (17,18). Phencyclidine enhanced ambulatory activity in all rats, and produced locomotor sensitization after daily injections. In contrast to phencyclidine,

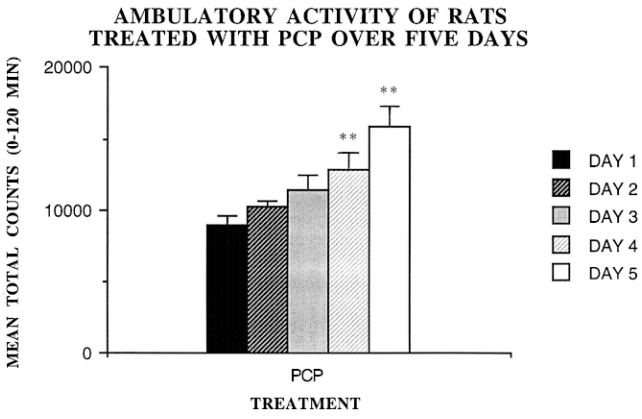

FIG. 3. Ambulatory activity of rats treated with phencyclidine over 5 consecutive days. Each bar represents the mean total counts for ambulation in 120 min ± SE for six rats. **$p < 0.01$ compared with activity on day 1.

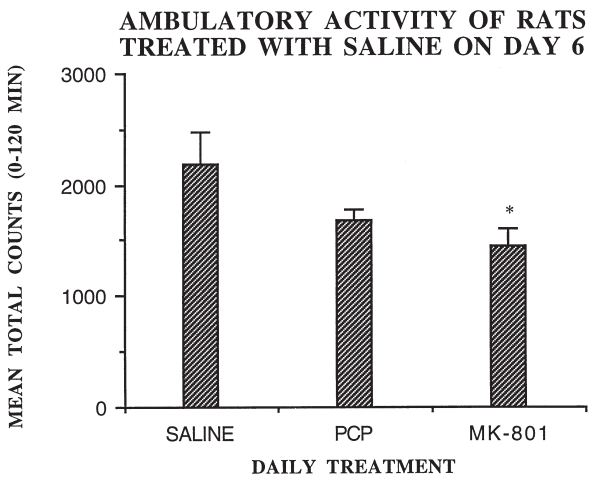

FIG. 4. Effect of saline on ambulatory activity of rats with different drug histories. Saline: rats received three prior daily saline injections. PCP: rats received five prior daily PCP injections. MK-801: rats received four prior daily MK-801 injections. Each bar represents the mean total counts for ambulation in 120 min ± SE for six to eight rats. *$p < 0.05$, compared with the saline group.

MK-801 produced only an enhanced locomotor stimulant effect in the majority of rats. In the present study, MK-801–sensitized rats showed cross-sensitization to phencyclidine, but phencyclidine-sensitized rats did not show cross-sensitization to MK-801. Thus, an asymmetric cross-sensitization between phencyclidine and MK-801 was observed.

When MK-801–sensitized rats received an injection of phencyclidine on day 5, they showed significant increases in ambulation (Fig. 2). If the increases in ambulation were due to injection–environmental conditioning, that is, if the MK-801–sensitized rats associated injection procedures in the experimental environment with increased ambulatory activity, these rats would also show increases in ambulatory activity following an injection of saline in the experimental environment. However, those same rats did not show any increases in ambulation when given an injection of saline on day 6 (Fig. 4). Interestingly, when given saline on day 6, daily MK-801–treated rats and daily phencyclidine-treated rats showed less locomotor activity than daily saline-treated rats. This phenomenon could be the result of an increased habituation to the injection procedures following repeated drug injections, because daily MK-801–treated rats and daily phencyclidine-treated rats received five prior daily injections, whereas daily saline-treated rats received three prior daily injections. Thus, cross-sensitization from MK-801 to phencyclidine cannot result from injection–environment conditioning.

Castellani and Adams (2) reported that rats developed sensitization to locomotor stimulant effects of phencyclidine after four daily injections, but the increase in locomotor activity is followed by a decrease to the day 1 level of locomotor activity following further daily injections. In the present study, phencyclidine-sensitized rats showed further increases in ambulatory activity following an injection of phencyclidine on day 5 (Fig. 3), although phencyclidine-sensitized rats did not show increases in ambulatory activity following an injection of MK-801 on day 5 (fig. 2). Thus, the failure of cross-sensitization from phencyclidine- to MK-801–induced ambulation cannot be due to reduced phencyclidine-like effects or other physical limitations of the rats on day 5.

The present results suggest that there are important differences in the in vivo neuronal mechanisms underlying repeated administration of MK-801 and phencyclidine. Because phencyclidine-sensitized rats did not show cross-sensitization to MK-801, it is unlikely that NMDA receptors play a major role in the development of phencyclidine sensitization. Reciprocal cross-sensitization of locomotion occurs between phencyclidine and racemic as well as (+) and (−) NANM (6,8). The (+) and (−) NANM enantiomers are ligands for multiple receptors. (±)NANM, in particular, has moderate to high affinity for sigma receptors (15). Furthermore, reciprocal cross-sensitization of locomotion occurs between phencyclidine and (−) pentazocine, a sigma ligand (20). Thus, phencyclidine-induced sensitization may be mainly mediated by an interaction with sigma receptors. Hence, although DA, NMDA, and sigma receptors can each be involved in behavior sensitization, PCP-induced sensitization may be a sigma-mediated effect.

In conclusion, although both phencyclidine and MK-801 enhanced ambulation, an asymmetric cross-sensitization occurred between phencyclidine- and MK-801–induced ambulation. Phencyclidine-sensitized animals did not show cross-sensitization to MK-801, whereas MK-801–sensitized animals showed cross-sensitization to phencyclidine, confirming our original observations (19).

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