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Effects of Caramiphen and Phencyclidine Alone and in Combination on Behavior in the Rat

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SZÉKELY, J. I., L. G. SHARPE AND J. L. KATZ. *Effects of caramiphen and phencyclidine alone and in combination on behavior in the rat.* PHARMACOL BIOCHEM BEHAV 47(3) 709–713, 1994. – Because dextromethorphan (DM) has been shown to inhibit the locomotor stimulant effects of phencyclidine (PCP), this study explored further the possible interaction between drugs acting on DM and PCP receptor sites. Caramiphen, an antitussive that binds with high affinity to the DM site, was injected (IP) alone (15–120 mg/kg) or at two doses (15 or 60 mg/kg) 15 min before a challenge dose of PCP (1.25–20 mg/kg). Caramiphen alone dose-dependently increased ataxia, increased stereotypy, and had no effect on locomotor activity. PCP alone dose-dependently increased ataxia, stereotypy, and locomotor activity, the latter showing an inverted U-shaped function. At both pretreatment doses, caramiphen enhanced locomotor activity and stereotypy when combined with low PCP doses but decreased these behaviors at high PCP doses. Caramiphen produced a dose-dependent additive effect on ataxia when combined with all PCP doses. It was concluded that, although caramiphen, like DM, inhibited the locomotor stimulant effects of selected doses of PCP, that interaction appeared to be due to other behaviors (e.g., ataxia/stereotypy) elicited by caramiphen combined with high doses of PCP. This study underscored the importance of using full dose ranges of PCP when attempting to antagonize its behavioral effects with other drugs.

Locomotor activity Stereotypy Ataxia Dextromethorphan Phencyclidine Caramiphen

PHENCYCLIDINE (PCP) is a dissociative anesthetic with a complex pharmacological profile. It is abused by humans, although it produces psychotomimetic effects (15,20). Animals self-administer PCP but its gross behavioral effects depend on the species being studied [for review see (24)]. In rodents, PCP effects are characterized by increased locomotion, stereotypy, and ataxia (10,14,21).

The neurochemical mechanisms responsible for mediating the behavioral effects of PCP are unclear. The mechanism most studied is its noncompetitive blockade of the *N*-methyl-D-aspartate (NMDA) receptor (2). While this mechanism appears to mediate many effects of PCP, including its anticonvulsant and behavioral effects, there is some evidence to suggest that many behavioral effects of PCP are unrelated to NMDA receptor blockade [for reviews, see (17,18,24)]. PCP also binds with intermediate affinity to a sigma receptor site [for reviews see (16,20)], which may account for some of its behavioral effects.

Dextromethorphan (DM) binds with high affinity to receptor sites distinct from those for PCP (3) and to sites related to two subtypes of sigma receptors (8). Interactions between PCP and sigma receptors (23) indicate that drugs similar to DM may antgonize the behavioral effects of PCP. Székely et al. (22) reported that DM inhibited the peak locomotor-stimulant effect of PCP (10 mg/kg) in a dose-dependent manner (22). However, because of the inverted U-shaped function of the PCP dose-effect curve (21), it is unclear whether DM antagonized PCP with a shift to the right in the ascending portion of the curve, or enhanced effects of PCP.

To further characterize this interaction we treated animals with caramiphen before several challenge doses of PCP. Caramiphen, like DM, is a nonopioid antitussive that binds with high affinity to the DM receptor site (3). We used caramiphen rather than DM for two reasons. First, it was necessary to complete the PCP dose-effect with the potential antagonist present, a procedure lacking in our previous study (22). Sec-

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ond, the active metabolite of DM, dextrorphan, binds with high affinity to the PCP site (12,19), produces PCP-like behavioral effects, and augments the behavioral effects of PCP (22), all of which would confound interpretation of the results. High doses of DM also produces PCP-like effects such as dysphoria and psychotomimetic effects in humans (7,13), probably due to its conversion to dextrorphan.

METHOD

Subjects

Male Sprague-Dawley rats (Harlan, Indianapolis, IN), 400–450 g at the start of the experiment, were grouped four per cage in a temperature- and humidity-controlled room (21 ± 1°C under a 12 L : 12 D cycle; lights on at 0800 h). Food and water were continuously available. The animals were maintained in accordance with the NIH Guide for Care and Use of Laboratory Animals (NIH/85-23).

Apparatus

Four activity chambers (photoelectric detectors 2.6 cm apart, Omnitech Electronics, Columbus, OH), measuring 42 × 42 × 45 cm, were used to record horizontal locomotor activity; stereotypy and ataxia were scored by observation [see below and (22)]. Horizontal activity was automatically recorded every 15 min for 60 min. White noise was used to mask extraneous noise.

Design and Procedure

All animals experienced two 1-h adaptation periods before test sessions with drugs began. A drug test session consisted of a pretreatment injection (IP, with saline or drug) followed 15 min later with a treatment injection (IP, with saline or drug). The animals were then placed singly in the activity chambers and observed continuously for 1 h. Stereotyped behavior was defined as the occurrence of any of the following eight behavioral signs typically elicited by PCP: forepaw treading, head weaving, hind limb abduction, back-pedalling, tremor, circling, gagging, and Straub tail (10). The degree of ataxia was rated in intensity from 0 to 5 (21). Every 15 min during the 1-h session, one observer (J.I.S.), under nonblind conditions, noted the presence or absence of the behavioral stereotypic signs and ataxia, thus forming maximum scores of 32 (8 × 4 time periods) and 20 (5 × 4 time periods), respectively. Each animal was observed within a 2-min period between the 5th and 10th min of each 15-min time period. Test sessions were separated by at least 7 days and were conducted between 1700 and 2000 h.

In Experiment 1 (caramiphen alone) the subjects (n = 16) received caramiphen in doses of either 15, 30, 60, or 120 mg/kg followed 15 min later by an injection of saline (a control for comparison with PCP injections in Experiment 2). To control for order effect, eight subjects received the four caramiphen doses in ascending order and the other eight in descending order. Control test sessions consisted of injecting saline followed by saline and were conducted twice in each subject as the first and last test session.

In Experiment 2 (caramiphen-PCP interactions), a different group of animals (n = 16) received a pretreatment injection of a low dose of caramiphen (15 mg/kg) or saline and were challenged 15 min later by one of five PCP doses (1.25, 2.5, 5, 10, or 20 mg/kg). Each animal received a total of 10 test sessions, the sequence of which consisted of alternate sessions with saline plus a PCP dose and caramiphen plus a PCP dose. To control for order effects, eight subjects received the challenge dose of PCP in ascending order, the other eight in descending order. The above experiment was repeated in another group of subjects (n = 16) except that the pretreatment dose of caramiphen was 60 mg/kg. Because there were no significant order effects, results were combined for presentation.

Two doses of caramiphen were used for interaction experiments with PCP. A low dose of 15 mg/kg was chosen because it has no effect on behavior maintained by food reinforcement (25). A higher dose of 60 mg/kg was chosen because of its ataxic effects (Fig. 1C) and because it is about twice the ED_{50} dose that significantly reduces lever pressing (25).

Drugs

Phencyclidine HCl (NIDA) and caramiphen edisylate (Sigma, St. Louis, MO) were dissolved in saline (0.15 M NaCl) and administered (IP) in a volume of 1 ml/kg body weight.

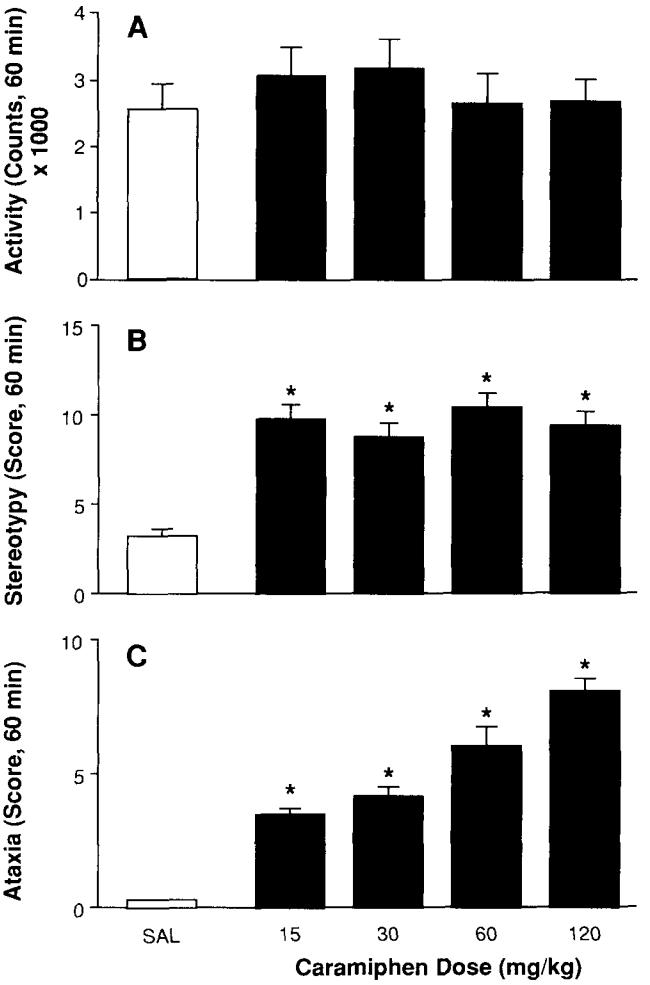

FIG. 1. Effects of caramiphen on locomotor activity (A), stereotypy (B), and ataxia (C). Caramiphen, injected 15 min before saline, had no effect on locomotion, increased stereotypy scores, and produced dose-dependent ataxia. Vertical lines = ± SE. *$p < 0.001$ compared with saline controls (S), Scheffé test (n = 16).

Data Analysis

For both experiments, the data for each behavior consisted of total scores over a 60-min time period after the treatment injection. Experiments 1 and 2 were analyzed with one- and two-way multiple analysis of variance (MANOVA), respectively. The Scheffé method was used to make a posteriori tests for paired comparisons. The level of significance was set at $p < 0.05$.

RESULTS

Experiment 1

Figure 1 shows the effects of caramiphen alone on locomotor activity, stereotypy, and ataxia. Caramiphen had no effect on locomotor activity, $F(4, 60) = 0.96$, but significantly increased stereotyped behavior at all four doses tested, $F(4, 60) = 28.4$, $p < 0.001$. However, the stereotypy produced by caramiphen, which consisted primarily of slight tremor and hind leg abduction, was not dose dependent. The extent to which the lack of graded stereotypy was due to method of scoring, high starting dose, or both, is unknown. Caramiphen, at all four doses, caused a significant dose-dependent increase in ataxia scores, $F(4, 60) = 94$, $p < 0.001$.

Experiment 2

Figure 2 shows the interaction of caramiphen with PCP. PCP alone (saline + PCP, open symbols) produced an inverted U-shaped dose-response curve for locomotor activity (Fig. 2A), $F(4,60) = 37.7$, $p < 0.001$. Although there was no significant difference between the 15 mg/kg CARAM + PCP vs. saline + PCP, $F(1, 15) = 0.45$, there was a significant leftward shift in the inverted U-shaped curve as indicated by the significant treatment × dose interaction, $F(4, 60) = 8$, $p < 0.001$. This was also reflected in the significant differences at 1.25 and 2.5 mg/kg dose of PCP. At the high caramiphen dose (60 mg/kg), the effects of lower PCP doses were also enhanced; however, the maximal effects of PCP were shifted downward: treatment effect, $F(1, 15) = 12.4$, $p < 0.005$; dose effect, $F(4, 60) = 27.2$, $p < 0.001$; treatment × dose interaction, $F(4, 60) = 17.1$, $p < 0.001$, respectively.

Given alone, PCP (saline + PCP) produced a dose-dependent increase in stereotypy (Fig. 2B) with significant linear regression ($r = 0.83$, $p < 0.01$). Pretreatment with caramiphen at both doses showed no consistent antagonistic action on any of the 8 PCP stereotypy signs analyzed separately. Therefore, the procedure of grouping these behaviors as a complex PCP profile (10) was used. The treatment main effects (saline + PCP vs. CARAM + PCP) were not significant, $F(1, 15) = 2.4$, and $F(1, 15) = 0.4$, for the 15 and 60 mg/kg group, respectively. The significant interaction effects, $F(4, 60) = 29.6$, $p < 0.001$, and $F(4, 60) = 42.5$, $p < 0.001$, for the 15 and 60 mg/kg group, respectively] are shown by the flattened apperance of the PCP dose-effect curve after caramiphen. The overall effect of caramiphen to reduce the slope of the regression line in the PCP dose-effect curve, characterized further caramiphen's dose-dependent action to enhance stereotypy at low PCP doses and to decrease these behaviors at high PCP doses ($r = 0.59$, $p = 0.05$ and $r = -0.04$, for the 15 and 60 mg/kg caramiphen group, respectively).

Like caramiphen alone (Fig. 1C), PCP alone (saline + PCP) caused a dose-dependent increase in ataxia (Fig. 2C). When combined with PCP, caramiphen appeared to produce an additive effect on ataxia by shifting slightly the PCP dose-effect curve to the left in a parallel manner: treatment effects, $F(1, 15) = 12.8$, $p < 0.005$, and $F(1, 15) = 100$, $p < 0.001$, for the 15 and 60 mg/kg caramiphen group, respectively; dose effects, $F(4, 60) = 191$, $p < 0.001$, and $F(4, 60) = 190$, $p < 0.001$, for 15 and 60 mg/kg caramiphen group, respectively; treatment × dose interaction: $F(4, 60) = 3.4$, $p < 0.05$, and $F(4, 60) = 0.07$, for the 15 and 60 mg/kg group, respectively.

DISCUSSION

In a previous study, we compared the interaction of several doses of dextromethorphan (DM) and dextrorphan with that of 10 mg/kg of PCP, a dose that elicits peak locomotor stimulant effects in rats (22). In that study, dextrorphan, but not DM, elicited behavior similar to PCP; DM dose-dependently inhibited the locomotor stimulant effects of PCP. However, the problem associated with the metabolic effects (dextrorphan) of DM combined with the lack of dose-effect analysis did not allow for unambiguous interpretation of the drug interactions. Because doses higher than 10 mg/kg PCP depressed behavior in general, we questioned the extent to which DM may enhance this suppressive effect or may antagonize effects of PCP by shifting the dose-effect curve to the right. Like DM, caramiphen, in the present study, also inhibited the locomotor stimulant effects of 10 mg/kg PCP. However, although caramiphen alone had no effect on spontaneous locomotion, it shifted the PCP dose-effect curve for locomotion slightly to the left when combined with PCP. Caramiphen enhanced the descending limb of the curve, especially by the 60 mg/kg dose which, when combined with the higher PCP doses (10 and 20 mg/kg), produced a downward shift in the curve. Caramiphen also suppressed the stereotypy produced by the higher PCP doses. These results indicate that caramiphen enhanced the behavioral effects of PCP.

Because the nature of the behavioral syndrome elicited by PCP is complex, it is difficult to determine the extent to which an elicited behavior interferes with the expression of another. Drugs that produce motor impairment and/or sedation could interact with psychomotor stimulants to inhibit locomotor activity and stereotypy (21). Although caramiphen appeared non-PCP-like, the behavior most similar to injections of caramiphen and PCP alone was dose-dependent ataxia. The additive-like effects of the caramiphen-PCP interaction on ataxia (Fig. 2C) may account, in part, for the apparent antagonism by caramiphen on locomotor activity and stereotypy at the higher PCP doses. However, when combined with the lower doses of PCP (1.25 and 2.5 mg/kg), caramiphen enhanced locomotor activity (Fig. 2A) even though given alone it had no locomotor stimulant action. Caramiphen elicited mild forms of stereotypy (hind limb abduction, tremor) which may have contributed to the enhanced locomotor activity when caramiphen was combined with low PCP doses which have been reported to produce stereotypy beginning with grooming, sniffing, and exploration (21). Conversely, the occurrence of more motionless patterns of stereotypy (e.g., forepaw treading, head weaving) could contribute to inhibition of locomotor activity at high PCP doses. Regardless of the behavioral interactions, the general effects of caramiphen to shift the PCP dose-effect curve slightly to the left is evidence that caramiphen enhanced rather than antagonized the effects of PCP. However, evidence to determine the involvement of DM selective receptors in the enhancement effects is lacking.

Results of this study emphasize the importance of using complete dose ranges of PCP in studies designed to antagonize

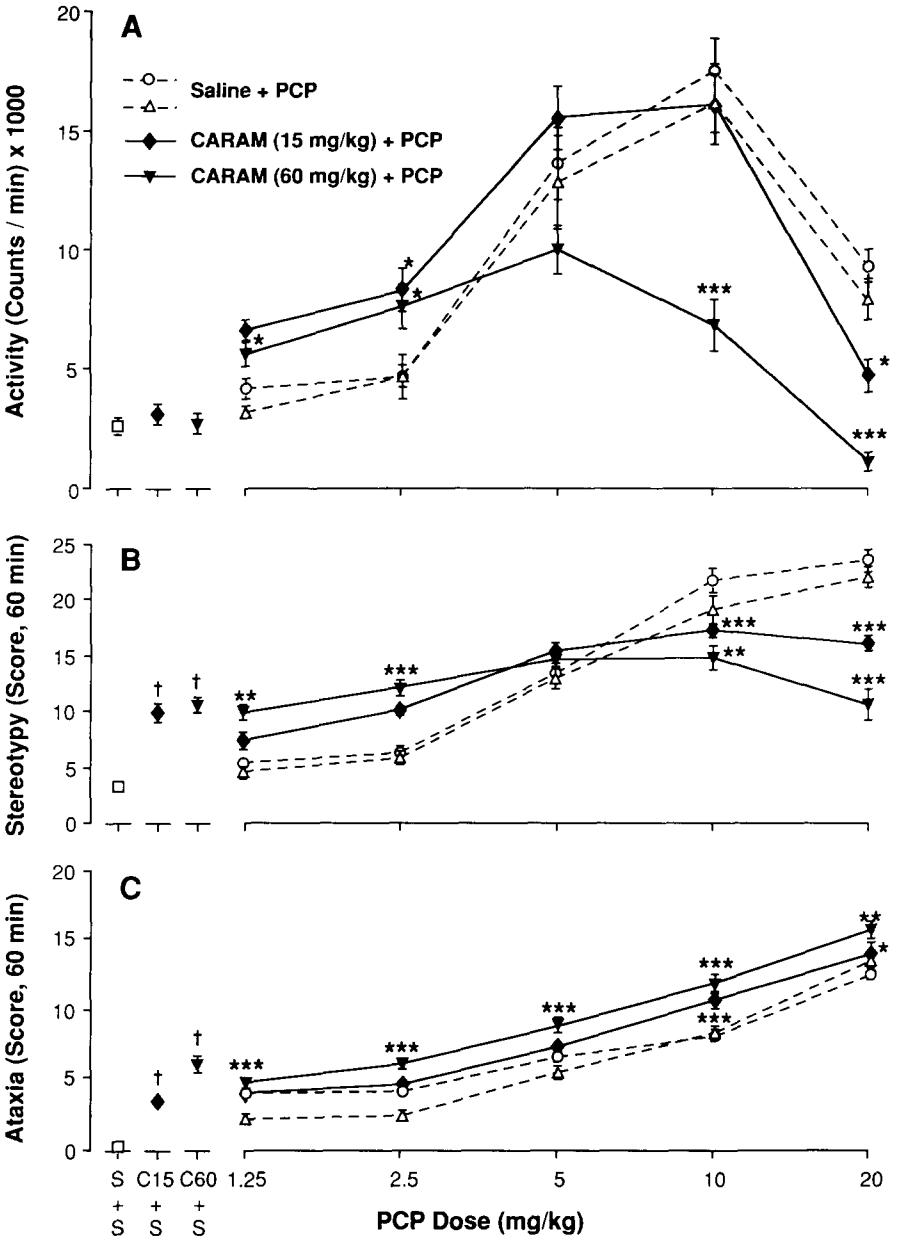

FIG. 2. Effects of caramiphen (CARAM) pretreatment (15 and 30 mg/kg) on behavior produced by PCP. Vertical lines = ± SE. *$p < 0.05$ **$p < 0.01$, ***$p < 0.001$, compared with respective saline + PCP groups, Scheffé test. †$p < 0.001$ compared with Saline (S) + S controls, Scheffé test. C15 and C60 = caramiphen doses of 15 and 60 mg/kg, respectively ($n = 16$ for each caramiphen-dose group).

psychomotor stimulant effects of PCP with other drugs. Drugs that effectively antagonize PCP-induced behavior should ideally shift the PCP dose-curve to the right in a parallel manner, a conclusion not yet demonstrated. Previous studies like ours (22) have examined one or two behaviorally active doses of PCP or PCP-like drugs (e.g., dizocilpine) in combination with several doses of proposed antagonist drugs (1,4–6,9–11,14,22). While such studies contribute toward the pharmacology of PCP, they risk inconclusive results about the interactions between PCP and other types of receptor. Further research is required to attribute with certainty the effects of caramiphen (or DM) or PCP to any particular binding site or receptor (10).

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