

PHENCYCLIDINE-LIKE BEHAVIORAL EFFECTS IN PIGEONS INDUCED BY SYSTEMIC
ADMINISTRATION OF THE EXCITATORY AMINO ACID ANTAGONIST, 2-AMINO-5-
PHOSPHONOVALERATE.

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Summary

A selective N-methyl-D-aspartate antagonist, DL-2-amino-5-phosphonovalerate, was found to produce PCP-like catalepsy, discriminative stimulus effects, and stereotyped operant responding in pigeons when administered intramuscularly. These results support the hypothesis that the behavioral effects of PCP-like drugs result at least in part from reduced neurotransmission at excitatory amino acid synapses utilizing N-methyl-D-aspartate preferring receptors.

Phencyclidine (PCP) - like drugs selectively antagonize excitation of spinal neurons by N-methyl-D-aspartate (NMDA) [e.g., 1,2] and inhibit NMDA-stimulated efflux of acetylcholine in rat striata [3]. The behavioral effects of PCP-like drugs might result from reduced neurotransmission at excitatory synapses utilizing NMDA preferring receptors. If this hypothesis is correct, drugs that are known to antagonize effects of NMDA should produce PCP-like behavioral effects.

Recently, we reported that intracerebroventricular (i.c.v.) administration of DL-2-amino-5-phosphonovalerate (AP5), a potent and highly selective NMDA antagonist [4], produces PCP-like catalepsy in pigeons [5]. In addition, the ability of NMDA-antagonists to induce PCP-like catalepsy appears to be correlated with their relative potency as NMDA antagonists [6]. Together, these findings constitute evidence for the mediation of a behavioral effect of PCP-like drugs by inhibition of neurotransmission at excitatory synapses utilizing NMDA preferring receptors. The present study examines whether other behavioral actions of PCP-like drugs may be mediated in a similar fashion.

First, we studied whether AP5, administered by intramuscular injection (i.m.), produces PCP-like catalepsy in pigeons. This experiment was done to study whether i.m. administration would produce behavioral effects similar to

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those observed previously after i.c.v. administration, and to establish the potency relation between these routes of administration. Second, the ability of AP5 (i.m.) to produce PCP-like discriminative stimulus effects was studied. Drug discrimination, a procedure which yields results of considerable pharmacological specificity, was used to investigate the similarity of AP5 to PCP with respect to interoceptive stimulus effects. Third, the ability of AP5 (i.m.) to produce PCP-like stereotyped responding was investigated. PCP induces grossly observable stereotypy in rats, but does not in pigeons; however, we have found PCP to increase the probability of repetition of non-reinforced responses in pigeons, as described herein, using an adaptation of an operant procedure that has been employed to study drug-induced stereotypy in rats [7].

Methods

Subjects. Fourteen White Carneaux pigeons were housed individually with water and grit freely available. Eight pigeons had continuous access to mixed grain. Six pigeons were maintained at 80% of their free-feeding weight by providing mixed grain in the home cage after each experimental session.

Catalepsy. This procedure has been described in detail elsewhere [8]. During an experimental session, drug injections were given i.m. (1 ml/kg) at 20-min intervals and effects (i.e., presence or absence of catalepsy, defined as loss of righting without head-drop and without eye closure) were assessed 15 min after each injection. Each injected dose (after the first) was calculated such that the total amount injected before each test was $\frac{1}{2}$ or $\frac{1}{4}$ log-unit greater than the previous dose. Cumulative dosing continued until a dose was reached that produced catalepsy in each subject. The time course of the drug effect was studied by tests at successive time intervals after the last injection. Four of the eight pigeons, which had free access to food, were tested with PCP. The remaining four pigeons were tested with AP5. The lowest dose which produced catalepsy (referred to as the catalepsy threshold dose) and the longest time interval after the last injection of the session at which catalepsy was present (in min), were determined for each pigeon.

Discriminative stimulus effects. Three food-deprived pigeons were trained to discriminate between an i.m. injection of PCP (1 mg/kg) and of saline, using operant conditioning chambers which contained two response keys and a hopper through which mixed grain was made available for reinforcement. Each daily session was preceded by an injection of either PCP or saline, after which the pigeon was placed in the experimental chamber. After 10 min, during which the chamber was dark and key-peck responses had no programmed consequences, the house-light came on and the left and the right key were illuminated red. Drug and saline sessions alternated according to a double alternation sequence. Twenty responses on the injection-appropriate key (i.e., left key PCP-appropriate, right key saline-appropriate) resulted in 4-sec access to mixed grain. Responses on the inappropriate key had no programmed consequences. A session ended after 50 reinforcements or 1 hr, whichever occurred first. When the training criterion was met (i.e., less than 5 responses on the inappropriate key, prior to the first food presentation, during 10 consecutive sessions), test sessions and training sessions were alternated. A test session ended when 20 responses had been made on either key or after 1 h, whichever occurred first. The key on which 20 responses accumulated first was defined as the selected key.

Tests were conducted only if the injection-appropriate key was selected during both the immediately preceding drug training session and saline training session, otherwise drug and saline training sessions were alternated until the appropriate key was selected during two consecutive sessions. The

percentage of PCP-key selections was used as a measure of PCP-like discriminative stimulus effects. The lowest dose that produced selection of the PCP key (generalization threshold dose) was determined for each subject, for each drug. Selection latency was defined as the time between illumination of the response keys and the occurrence of key selection. Each dose of PCP and of AP5 was tested twice. AP5 was tested using an injection-test interval of 30 min; PCP was evaluated 10 min after its administration.

Stereotypy. An operant procedure used to study drug-induced stereotypy in rats [7] was adapted for use in pigeons. Three food-deprived pigeons were trained to respond in an operant chamber. After 10 min, during which the chamber was dark and responses had no programmed consequences, the left and the right key were illuminated red. One of the two keys was selected randomly ($P = 0.5$) to provide 3-sec access to grain following a key peck. Pecking the "unselected" key had no programmed consequences. After food had been presented, the computer program selected randomly which key would provide reinforcement next. The session ended after 60 reinforcements or after 1 hr, whichever occurred first.

After performance had stabilized, every session was preceded by an injection of either saline (S) or drug (D) according to the following, weekly repeating, sequence: S-D-S-D-S-D-S. Data obtained during the first saline session of each sequence were used to calculate control values. The probability that a response was repeated on the key "unselected" for food delivery was used as a measure of stereotyped responding and was calculated by dividing the number of repetitions by the sum of repetitions of, and switches between, left and right key responses. Rate of responding was measured as total number of responses divided by session length. Each dose of AP5 was tested twice, using an injection-test interval of 30 min; PCP was evaluated 10 min after its administration.

Statistical analysis. Data were analyzed by Student's t-test and by one-factor repeated measures ANOVA [9]. Post-hoc comparisons of particular doses with control were made using Dunnett's t-test. Threshold doses and selection latencies were log-transformed prior to statistical analysis.

Drugs. The compounds used were phencyclidine base (Warner-Lambert/ Parke-Davis and Co., Ann Arbor, MI) and DL-2-amino-5-phosphonovalerate hydrochloride (Dr. P. Ornstein). PCP was dissolved in sterile water to which a small amount of lactic acid was added. AP5 was dissolved in a minimum quantity of 1N NaOH, to which sterile water was added. Doses of drugs are expressed in the forms described above.

Results

AP5 produced PCP-like catalepsy when administered i.m. (Fig. 1, upper panel). The mean catalepsy threshold dose of AP5 (± 1 S.E.M.) was 195 ± 46 mg/kg, which is about 120 times larger than the mean catalepsy threshold dose of PCP (i.e., 1.64 ± 0.58 mg/kg) ($t = 11.02$, $df = 6$, $P < 0.001$). The duration of the AP5-induced catalepsy was significantly longer than the duration of the cataleptic effects of PCP (420 ± 69 min and 83 ± 23 min, respectively; $t = 4.64$, $df = 6$, $P < 0.01$).

PCP (1 mg/kg) acquired discriminative control over responding in all three pigeons, as evidenced by a dose-dependent effect on the percentage of PCP-key selections (Fig. 1, middle panel; mean generalization threshold dose: 0.72 ± 0.16 mg/kg). PCP did not significantly affect selection latency ($F[4,8] < 1.0$; overall mean latency 10.9 ± 4.5 sec). AP5 produced PCP-appropriate responding at a mean generalization threshold dose of $139 \pm$

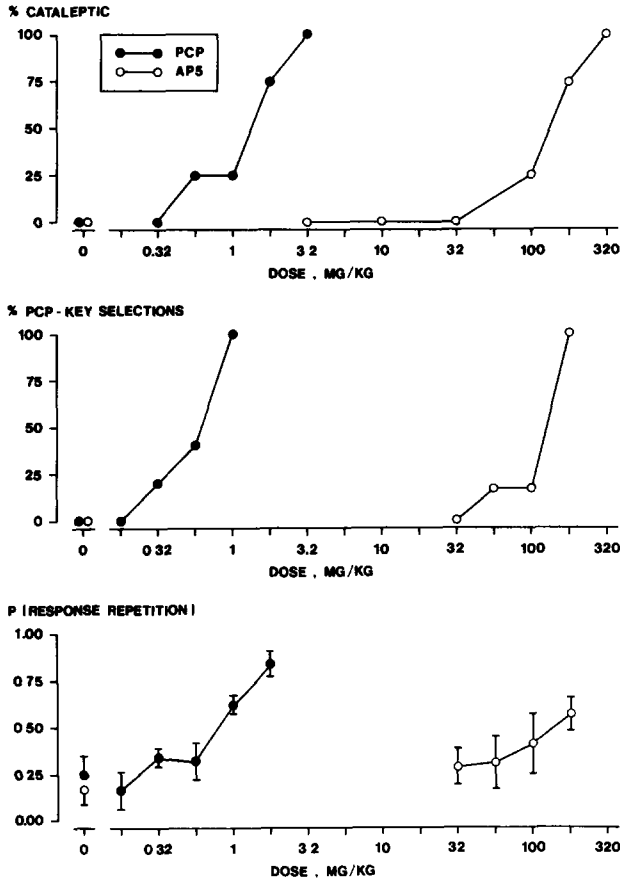


Fig. 1

Upper panel: dose-response curves of PCP- and AP5-induced catalepsy (loss of righting without head-drop and without eye closure) in pigeons (n=4 per drug). Ordinate: percentage of subjects showing catalepsy. Abscissa: cumulative i.m. dose administered. Middle panel: discriminative stimulus effects of PCP and of AP5 in pigeons (n=3) trained to discriminate between i.m. injections of PCP (1 mg/kg) and of saline. Each dose was tested twice. Ordinate: percentage of PCP-key selections. Abscissa: i.m. dose administered. Lower panel: stereotyped key pecking, ascertained by measuring the probability that non-reinforced responses are repeated after i.m. administration of PCP and of AP5, in pigeons (n=3). Each dose of AP5 was tested twice; each dose of PCP was tested once. Ordinate: probability of repetition of non-reinforced responses. Abscissa: i.m. dose administered. Dose-response curves of PCP-induced discriminative stimulus effects and of PCP-induced stereotyped responding are replotted from other sources (Koek et al., *Metaphit*, a proposed phencyclidine receptor acylator: PCP-like behavioral effects and evidence of absence of antagonist activity in pigeons and in rhesus monkeys, *J. Pharmacol. Exp. Ther.* 237:386-392, Fig. 3).

41 mg/kg. The generalization threshold dose was about 190 times higher than the generalization threshold dose of PCP ($t = 8.63$, $df = 2$, $P < 0.02$). AP5 significantly increased selection latency ($F[3,6] = 26.01$, $P < 0.001$): the mean selection latency was significantly increased to 142 ± 65 sec after 100 mg/kg of AP5 ($P < 0.05$), and to 1871 ± 349 sec after 180 mg/kg.

PCP increased the probability of repetition of non-reinforced responses (Fig. 1, lower panel; $F[5,10] = 17.85$, $P < 0.001$); the probability of response repetition was increased significantly by 1 mg/kg of PCP ($P < 0.01$) and by 1.8 mg/kg of PCP ($P < 0.01$). The overall rate of responding was 0.40 ± 0.02 responses/s after administration of saline. PCP decreased the overall rate of responding ($F[5,10] = 7.20$, $P < 0.005$); response rate was reduced to 0.21 ± 0.06 responses/s by 1.8 mg/kg of PCP ($P < 0.01$). The AP5-induced increase of response repetition approached statistical significance ($F[4,8] = 3.64$, $P = 0.057$); response repetition was increased by 180 mg/kg of AP5 ($P < 0.05$). The overall rate of responding was not affected by AP5 ($F[4,8] = 1.52$, $P > 0.20$).

Discussion

AP5, a selective NMDA antagonist, produces PCP-like behavioral effects in pigeons. In a previous study, AP5 was found to induce PCP-like catalepsy in pigeons when administered i.c.v. at a dose of 1 μ mol [5]. PCP-induced catalepsy has been shown to be pharmacologically specific and stereoselective, as evidenced by the observation that pentobarbital fails to induce catalepsy and that catalepsy is induced by the (+)-isomers but not by the (-)-isomers of PCP-like drugs [8]. The observation that AP5 produced PCP-like catalepsy supports the suggestion that NMDA-antagonism may underly the cataleptic effects of PCP-like drugs. AP5 produced PCP-like catalepsy in all pigeons, when administered i.m. in a dose of 320 mg/kg. Therefore, AP5 appears to be about 700 times less potent when administered i.m. as compared to i.c.v. administration. This large potency difference is compatible with the finding that AP5 and related compounds cross the blood-brain barrier relatively poorly, and is similar to the potency ratio between i.c.v. and i.p. administered AP5 in blocking convulsions in mice [10].

AP5 produced PCP-appropriate discriminative responding in all pigeons when administered i.m., and was about 190 times less potent than PCP. In pigeons, PCP-like drugs induce discriminative stimulus control that is distinct from pentobarbital and a variety of other drugs [11,12,13 and unpublished observations]. The result of the present study suggests that NMDA antagonism may underly the discriminative stimulus effect of PCP in pigeons.

PCP induces stereotypy in rats. A procedure used to measure drug-induced stereotyped operant responding in rats [7] was adapted for use in pigeons and was found to be sensitive to the effects of PCP. PCP and AP5 increased response repetition, which suggests that these drugs induced stereotyped operant responding. Drug-induced response repetition in this procedure is to some extent pharmacologically selective, in that response repetition was increased also by apomorphine, d-amphetamine, and pentobarbital, but was not affected by behaviorally active doses of chlordiazepoxide, scopolamine, morphine, chlorpromazine and haloperidol [unpublished observations].

The results suggest that PCP produces discriminative stimulus effects and stereotyped responding at doses which are only marginally effective in producing catalepsy (Fig. 1). However, AP5-induced discriminative stimulus effects, stereotyped responding and catalepsy appear to occur within a similar dose-range. This difference between PCP and AP5 deserves further

study because it may mean that NMDA-antagonists produce behavioral effects only at doses very close to the cataleptic (and possibly anesthetic) dose, unlike PCP-type drugs.

In conclusion, the NMDA-antagonist AP5 produced PCP-like catalepsy, discriminative stimulus effects and stereotyped operant responding in pigeons. These results support the hypothesis that a reduction of neurotransmission at excitatory synapses utilizing NMDA receptors underlies certain behavioral effects of PCP in pigeons. One of the implications of this hypothesis is that NMDA antagonists might have PCP-like psychotomimetic and anesthetic actions and potentially could yield a new class of anesthetic agents.

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