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The Reinforcing and Discriminative Stimulus Properties of Para-Ethoxy- and Para-Methoxyamphetamine

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CORRIGALL, W. A., J. M. ROBERTSON, K. M. COEN AND B. A. LODGE. *The reinforcing and discriminative stimulus properties of para-ethoxy- and para-methoxyamphetamine.* PHARMACOL BIOCHEM BEHAV **41**(1) 165–169, 1992.—The purpose of this study was two-fold: 1) to assess the degree to which para-methoxyamphetamine and para-ethoxyamphetamine maintain self-administration behavior, and 2) to determine the similarity or difference between these drugs and amphetamine in drug discrimination tests. Animals were trained to self-administer 0.3 mg/kg/infusion cocaine on a fixed-ratio 5 (FR5) schedule of reinforcement. Substitution of para-ethoxyamphetamine (PEA), para-methoxyamphetamine (PMA), or saline produced similar results; in all cases responding decreased substantially. A separate group of animals was trained to discriminate amphetamine (1 mg/kg) from saline in a fixed-ratio (FR10), food-reinforced paradigm. PEA and PMA produced only limited responding on the amphetamine-appropriate lever (maximum of approximately 30%). Both PMA and PEA had effects on response rate which were similar to those of amphetamine, although PMA had slightly greater rate-decreasing effects than the other two compounds. These data suggest that neither PMA nor PEA are reinforcing in rats, and do not possess amphetamine-like discriminative properties.

Para-ethoxyamphetamine Drug discrimination Para-methoxyamphetamine Reinforcement Self-administration

ALTHOUGH amphetamine tends to produce predominantly stimulant effects on the central nervous system, the 4-methoxy-substituted derivative (para-methoxyamphetamine, PMA) appears to produce both stimulant and hallucinogenic effects [e.g., (8)], and to have no positive reinforcing properties in an animal self-administration model (4,11). The related substance, 4-ethoxyamphetamine (para-ethoxyamphetamine, PEA), appeared in Canada in 1987 as a product of a clandestine laboratory. Because of its structural similarity to PMA, there is considerable interest among health authorities in determining the behavioral pharmacological profile of this drug.

At present there are no studies of either the reinforcing or the discriminative stimulus properties of PEA. This study was carried out to determine 1) whether PEA produces amphetamine-like subjective effects, and 2) whether PEA will maintain self-administration behavior in an animal model. For these experiments, PMA was used as a comparison drug.

METHOD

Self-Administration Studies

Subjects were male Long-Evans rats (Charles River, Lachine, Quebec) drug naive at the time experiments were begun. Animals were housed in hanging wire cages in a reversed light-dark cycle room (lights off between 700 and 1900 hours), and were initially maintained under ad lib feeding conditions.

After habituation to the colony room, animals were deprived of food for a period of 24–48 hours and trained to press a lever on a schedule of continuous reinforcement in order to receive 45 mg food pellets (Precision Pellets, BioServ, Frenchtown, NJ). This training was provided to enhance the initial rate of drug self-administration, i.e., to familiarize the animals with operant responding. When training to respond for food was completed, animals were returned to a regular feeding schedule consisting of the daily nutritional requirement of rat chow (20 g) provided as a single meal.

For drug self-administration experiments, each animal was surgically prepared with a chronic intravenous (IV) catheter. Surgery was performed under anesthesia produced by intraperitoneal (IP) injection of acepromazine maleate (10 mg/kg), followed approximately 10 minutes later by intramuscular injection of ketamine hydrochloride (100 mg/kg). A recovery period of 3–7 days occurred following surgery and prior to further training.

After recovery from surgery, animals were allowed to acquire self-administration of cocaine hydrochloride. These experiments were performed identically to other studies from this laboratory

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[e.g., (1–3)]. Self-administration sessions were carried out in chambers equipped with two levers; responding on one of the levers resulted in drug delivery to the animal when schedule requirements were met, whereas responding on the other lever was recorded but not reinforced. Animals initially had access to cocaine on a CRF schedule. Response requirements were increased over a two-week period to a final value of FR5, i.e., 5 presses on the lever were required to produce a drug infusion. Following each infusion there was a 1-minute time-out period during which responding was recorded but not reinforced by drug delivery. The dose of cocaine used for self-administration was 0.3 mg/kg/infusion; this dose was chosen since it is midrange for cocaine self-administration (3). Drug self-administration sessions were 1 hour in duration.

The ability of the substituted amphetamines to maintain self-administration behavior was tested when responding for cocaine had stabilized. For experimental manipulations, animals were randomly divided into 4 squads. Each squad was assigned to one of 4 dose levels of para-ethoxyamphetamine (0, 0.03, 0.1 or 0.3 mg/kg/infusion). Doses of substituted amphetamines for intravenous administration were chosen to be approximately 10-fold lower than the dose range which has been used for intraperitoneal administration [e.g., (5)]. Following tests of PEA, animals were given access to cocaine at 0.3 mg/kg/infusion again. Subjects were randomly reassigned to 4 squads, each to be tested with one dose level of para-methoxyamphetamine (0, 0.03, 0.1 or 0.3 mg/kg/infusion). Prior to testing each drug, animals with catheters of questionable patency were dropped from the experiments. In addition, only subjects whose catheters remained patent for the duration of the test period for each amphetamine were included in the analyses.

Drug Discrimination Studies

Subjects were of the same strain as those used for self-administration experiments, and were maintained in similar conditions. These animals were also trained to respond for 45 mg food pellets on a CRF schedule. Once subjects had learned to press a lever to obtain food, schedule requirements were increased promptly to FR10 and discrimination training was begun. Animals were given a daily injection of either saline (1 ml/kg IP) or amphetamine (1 mg/kg IP; same volume as saline) 15 minutes prior to the start of each daily operant session. Animals were trained to produce 10 consecutive responses on the left-hand lever after saline injections and the same fixed ratio on the right-hand lever after amphetamine, in order to receive food pellets. The schedule requirement was such that responding on the incorrect lever reset the requirement for the correct lever, that is, each subject was required to complete an uninterrupted FR10 on the correct lever. However, all responses were used to calculate response rate and the percentage of drug appropriate responding. Session duration was 15 minutes. To eliminate possible olfactory cues, consecutive animals running in the same operant chamber received opposite training injections on some days and the same training injections on others. Choice of saline or amphetamine as the training injection was made according to a predetermined sequence which repeated every 4 weeks.

A subject was considered to be trained to criterion level when it made no more than 2 incorrect responses before delivery of the first food pellet, and in addition, at least 90% of its total responding in the session was made on the correct lever. When these conditions were met, testing was begun.

Test sessions were carried out on Tuesdays and Fridays, subject to sustained training criteria on intervening days. On test days, both levers were active, and every uninterrupted FR10 on either lever resulted in the delivery of a food pellet. During this phase, training sessions were carried out on intervening nontest days. Doses for the test drugs were chosen by comparison with previous drug discrimination research with PMA [e.g., (5)].

Drugs and Solutions

The following drugs were used: cocaine hydrochloride (B.D.H., Toronto), and d-amphetamine sulphate, 4-methoxyamphetamine hydrochloride and 4-ethoxyamphetamine hydrochloride (all from the Bureau of Drug Research, Health and Welfare Canada, Ottawa). The purity of the amphetamine compounds was as follows: for d-amphetamine sulphate, $99^{+}\%$; for 4-methoxy amphetamine, $98^{+}\%$; for 4-ethoxyamphetamine, $98^{+}\%$. For self-administration, drug solutions were prepared with isotonic saline and were passed through a 0.22 micrometer filter prior to use. Each IV infusion was 0.1 ml/kg in volume, delivered in approximately 1 s. For drug discrimination, solutions were prepared in sterile isotonic saline and injected at a volume of 1 ml/kg. All doses refer to the base.

Analysis

Data in all cases are presented as the group mean, and bars show the standard error of the mean. The sample sizes are indicated in the figure captions.

For self-administration, statistical analyses of the drug substitution data were carried out using a within/between or split-plot design. Data from the two cocaine sessions prior to substitution and from the three substitution sessions were included in these analyses.

Response patterns during substitution were also examined to determine whether PMA or PEA resulted in temporal changes different from those of saline. To do this, the cumulative number of lever presses in each 1-minute time period were averaged across subjects. Average cumulative records for PMA or PEA substitution were compared with response patterns after saline substitution.

For drug discrimination, data from animals which did not complete at least one FR10 during testing were not included in calculating the drug appropriate response score; the number of animals not completing at least one FR10 at any dose is shown adjacent to the relevant data point in each figure. Data from all animals were of course used in calculating response rate.

RESULTS

Reinforcing Properties

Data from the PEA substitution experiment are shown in Fig. 1a. Cocaine maintained a high degree of self-administration behavior, with each of the four squads of animals responding to receive, on average, approximately 20 infusions of the drug in each daily 1-hour session. When PEA or saline vehicle were substituted for cocaine, the number of drug infusions obtained by the subjects decreased progressively over sessions such that by the third substitution session there was no difference between saline and any of the doses of PEA, $F(3,31)=0.80$, n.s. However, there was a significant effect of session during substitution, $F(4,124)=79.92$, $p<0.0001$, but no dose-by-session interaction, $F(12,124)=0.95$, n.s.

Data for PMA substitution are shown in Fig. 1b. There was little difference between the data for PMA and PEA. During PMA substitution, the number of infusions obtained by the animals again decreased rapidly over three sessions, and there was a significant effect of session, $F(4,100)=133.99$, $p<0.0001$,

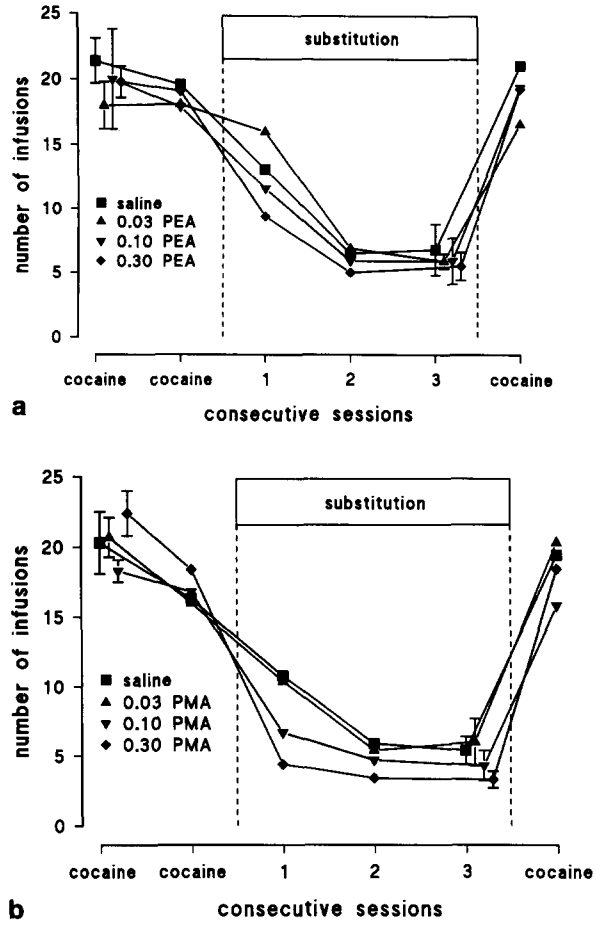

FIG. 1. Average number of drug infusions received by subjects in daily one hour sessions during cocaine self-administration at 0.3 mg/kg/infusion and during substitution of PEA (a) and of PMA (b). For clarity, not all error bars are shown on the points; irrespective of whether error bars are shown, however, each point represents the average across subjects but within a given session. For each group, self-administration data are shown for 1) two sessions prior to substitution in which cocaine was available, 2) for three substitution sessions, and 3) for a single postsubstitution session in which cocaine was again available. Substitution sessions are labelled 1, 2 and 3. For PEA, sample sizes are as follows: saline, n=8; 0.03 mg/kg/infusion PEA, n=9; 0.10 mg/kg/infusion PEA, n=8; 0.30 mg/kg/infusion PEA, n=10. For PMA, sample sizes are as follows: saline, n=8; 0.03 mg/kg/infusion PMA, n=7; 0.10 mg/kg/infusion PMA, n=6; 0.30 mg/kg/infusion PMA, n=8.

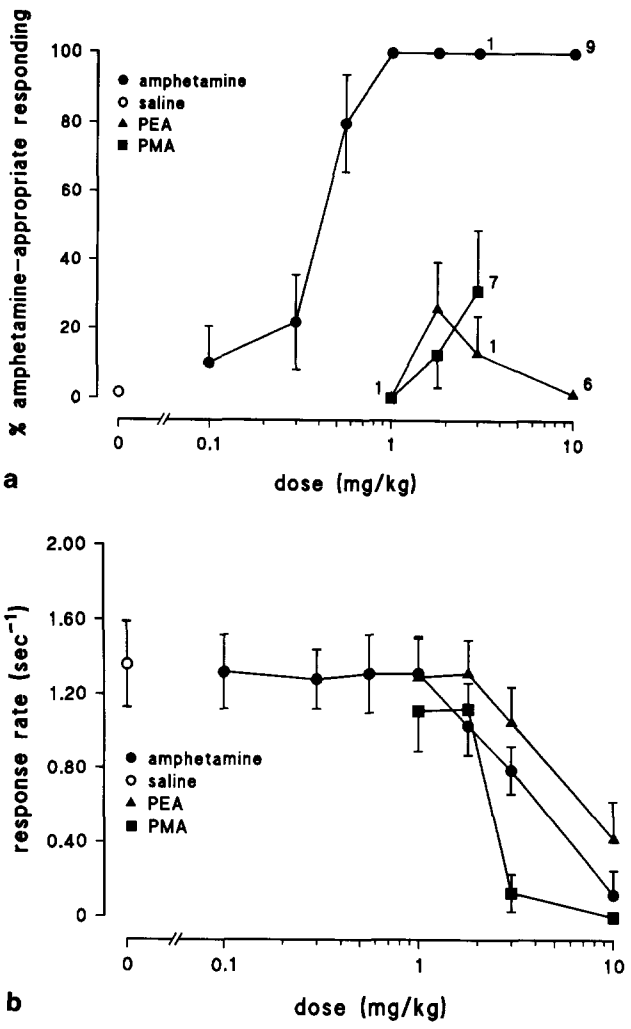

FIG. 2. Data for drug discrimination experiments (n=10). (a) Selection of the amphetamine appropriate lever by animals following different doses of amphetamine, PEA or PMA. A value beside a data point indicates the number of animals that did not complete at least one FR10 at that dose during the 15-minute session. PMA was tested at a dose of 10 mg/kg, but none of the 10 animals completed even a single FR10 at this dose. (b) Response rate for the same sample as a function of dose for amphetamine, PEA or PMA. Total responding (i.e., on both levers) was used to calculate the response rate.

but not of dose, $F(3,25)=0.90$, n.s. With PMA, there appeared to be a greater separation with dose, particularly on the first day of substitution. This is reflected in a significant dose-by-session interaction, $F(12,100)=2.00$, $p<0.05$.

Response patterns, as determined from averaged cumulative records, changed similarly irrespective of whether saline, PEA or PMA were available (data not shown). In addition, responding on the inactive lever was virtually absent during both cocaine self-administration and substitution tests (again, the data are not shown).

Discriminative Stimulus and Response-Decreasing Properties

Data for drug discrimination are shown in Fig. 2. For amphetamine, maximal selection of the drug-appropriate lever occurred at the training dose of 1 mg/kg; at this dose, response rate was not different from the response rate following saline treatment. At doses of amphetamine higher than 1 mg/kg, selection of the drug appropriate lever remained at 100%, but response rate decreased until, at 10 mg/kg, only 1 of 10 subjects completed a full FR10 on either lever.

Neither of the substituted amphetamines engendered substantial amphetamine appropriate responding at any dose (the maximum was not greater than approximately 30%). At 1 mg/kg, there was no responding on the drug appropriate lever. At higher doses, PEA and PMA produced only partial generalization to the amphetamine cue; PEA produced a maximum of 26% amphetamine appropriate responding at a dose of 1.8 mg/kg, whereas PMA produced similar generalization (31%) to the amphetamine cue at a dose of 3 mg/kg. At 10 mg/kg, PEA produced a sub-

TABLE 1

AVERAGE TOTAL RESPONSES ON BOTH LEVERS LEADING TO DELIVERY OF THE FIRST FOOD REINFORCEMENT IN DRUG DISCRIMINATION TESTS

Dose (mg/kg)	Amphetamine	PMA	PEA
0	10.5 (0.3)		
0.1	10.3 (0.3)		
0.3	10.8 (0.8)		
0.56	10.0 (0)		
1.0	10.0 (0)	10.3 (0.4)	11.1 (0.8)
1.8	10.0 (0)	10.1 (0.1)	10.6 (0.4)
3.0	10.0 (0)	13.3 (3.0)	10.7 (0.4)
10.0	10.0*	†	11.0 (1.2)

A value of 10 indicates that all subjects in the group selected a single lever and completed an uninterrupted FR10 on it to receive the first food reinforcement. Numbers in parentheses are SEM's. Sample sizes are as in Fig. 2.

*Only one subject completed an FR10 after this dose.

†No subjects completed the first FR10 after this dose.

stantial decrease in response rate and virtually no amphetamine-like responding. At 10 mg/kg PMA, none of the 10 subjects completed the first fixed ratio.

In terms of initial lever selection during testing, subjects either responded almost entirely on one or the other lever, or responded essentially not at all. That is, if subjects did not receive even the first food pellet during testing, it was generally because they responded only a few times, and then stopped for the remainder of the session, rather than because they continued to alternate between levers, completing less than an FR10 on each and resetting the response requirement. This observation is of course somewhat anticipated by the fact that low rather than high response rates accompany failures to complete the first FR10 (compare the number of animals not completing a single FR10 in the upper panel of Fig. 2 with the response rates in the lower panel). When the first FR10 was completed, rats generally did so without alternating between levers; Table 1 shows the average total responses on both levers leading to the delivery of the first food pellet. Note that a value of 10 indicates that all animals in the group selected a single lever, whether amphetamine or vehicle appropriate, and completed an uninterrupted FR10 on it. For amphetamine, these data show clearly that the animals were trained to chain responses in uninterrupted FR10 sequences. For the substituted derivatives, there is no marked deviation from this behavior, although at the 3 mg/kg dose of PMA one of the four animals responded on both levers to some degree. Overall these data show that the substituted amphetamines did not alter the ability of the animals to chain responding in sequential FR10's.

Although we did not systematically assess the effects of the amphetamines visually, casual observation of the animals prior to drug discrimination sessions showed that the training dose of amphetamine increased locomotor activity, including rearing, and produced some stereotypy. Rearing behavior occurred initially after 10 mg/kg amphetamine, but movements progressively became less coordinated with time. PEA and PMA generally decreased motor activity; at the highest doses tested animals' behavior appeared to be depressed.

DISCUSSION

The present data suggest that both PEA and PMA have limited ability to reinforce self-administration behavior in rats. This conclusion is based on the observation that the drugs did not maintain responding different from that of saline in substitution tests. When substituted for cocaine on a FR5 schedule, saline, PEA and PMA all occasioned a progressive decrease in the extent of responding, with a corresponding reduction in the number of infusions obtained. For PMA, the data are in agreement with a report that the drug is not self-administered by baboons (11), and maintains self-administration only transiently in drug naive rats (4). There have been no previous studies which have reported on the reinforcing properties of PEA. The present data provide the first information relevant to this issue, and suggest that the reinforcing properties of PEA are limited, similar to those of PMA.

Conclusions about the abuse liability of PEA in humans based on self-administration studies in animals should be made with caution, however. Whereas there is good concordance for drugs such as stimulants between self-administration data in animal studies and likelihood of use by humans, animal studies may be less relevant in predicting dependence potential in the case of drugs with hallucinogenic profiles (6). PMA and PEA may fall into this latter class. Furthermore, although animal studies show that PMA is not self-administered, use of the drug by humans clearly has occurred.

An additional caution should also be highlighted. We used substitution tests to investigate the reinforcing properties of PMA and PEA, rather than testing whether the drugs would initiate self-administration in naive subjects. It is possible that various factors, for example, contrast effects, might contribute to the absence of evidence for reinforcing properties in substitution tests. These data therefore do not rule out the possibility that PEA or PMA might support acquisition of self-administration behavior in rats. However, previous work by Davis et al. (4) has shown that PMA will maintain self-administration for a maximum of several days at a dose of 0.025 mg/kg/infusion. Since higher doses produced an immediate decline in responding, it may be that PMA is aversive in rats, but that several exposures are necessary for the aversion to manifest itself at low doses. Alternatively, the biphasic pattern in responding in Davis et al. may indicate development of some form of behavioral toxicity.

It should also be noted that self-administration tests in our study were not counterbalanced across PMA and PEA. We cannot exclude therefore the possibility that substitution of PEA might have had consequences for the subsequent PMA tests. However, the reassignment of subjects to test groups following the first substitution reduces the potential impact of any order effects.

With respect to discriminative stimulus properties, this research shows that PEA and PMA have little similarity to amphetamine, producing a maximum of approximately 30% generalization to the amphetamine cue. For PEA, maximal generalization occurred at 1.8 mg/kg, a dose at which response rate was not decreased. For PMA, however, maximal amphetamine responding occurred at 3 mg/kg, a dose at which response rate was markedly decreased. Previous studies with PMA have similarly shown partial, although somewhat higher, amphetamine appropriate responding. For example, Glennon et al. (5) reported that PMA produced a maximum of 60%–62% amphetamine appropriate responding at 1.8–2.0 mg/kg. Amphetamine appropriate responding decreased at both higher and lower doses, with essentially complete response disruption at a dose of 2.5 mg/kg. Huang and Ho (7) found approximately 80% responding on the drug appropriate lever after 2 mg/kg PMA in rats trained to discriminate amphetamine from saline.

Differences in the extent of generalization between these previous studies and the present one may be due to a variety of

factors, but most likely to differences in operant schedule or in the dose used to train discrimination. Previous studies have used either 0.8 or 1 mg/kg of amphetamine sulphate (i.e., as the salt) as the training dose. In the present study, the training dose was 1 mg/kg amphetamine sulphate, calculated as the base (equivalent to 0.68 mg/kg as the salt). The lower training dose employed in the present study may mean that animals were trained to a somewhat different set of drug cues, ones that allow less generalization to para-substituted amphetamines of this kind. In addition, neither of the studies noted above employed a fixed-ratio schedule; a DRL (differential reinforcement of low response rate) schedule was used by Huang and Ho, and a VI (variable interval) schedule was used by Glennon and coworkers. Schedule differences may therefore also contribute to the differences that have been observed in the extent of generalization of the amphetamine cue to PMA.

Unlike amphetamine, PMA appears to have prominent effects on the serotonin system. For example, PMA has been reported to result in the release of 5-HT (10), and some PMA effects can be attenuated by inhibition of 5-HT synthesis or receptor blockade (9). However, other studies of transmitter release suggest that mono-methoxy-substituted phenylisopropylamines might be capable of producing both amphetamine-like and LSD-like effects (12,14), perhaps accounting for observations that PMA has both amphetamine-like and LSD-like behavioral profiles (8). Furthermore, a direct involvement of 5-HT in PMA effects has not been supported by drug discrimination studies (14). For PEA, it is not yet known to what extent its effects are due to actions on serotonin or on other neurotransmitter systems. Irrespective of the mechanism(s) through which PEA and PMA act, the present study suggests that their behavioral pharmacology is not similar to that of amphetamine; they produce little amphetamine-like responding in drug discrimination tests, and do not maintain responding greater than that of saline in a self-administration paradigm.

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