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Discriminative Stimulus Properties of Triadimefon: Comparison With Methylphenidate[1]

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PERKINS, A. N., D. A. ECKERMAN AND R. C. MACPHAIL. *Discriminative stimulus properties of triadimefon: Comparison with methylphenidate.* PHARMACOL BIOCHEM BEHAV **40**(4) 757–761, 1991.—Two groups of rats (N=4 each) were trained to discriminate either triadimefon (40 mg/kg) or methylphenidate (4 mg/kg) from saline in a two-lever, milk-reinforced drug discrimination paradigm. Dose-response functions were determined during 5-min extinction sessions. Both agents produced a dose-related increase in the percentage of responses that occurred on the drug lever. In the substitution phase of the study, rats trained to discriminate triadimefon were tested with methylphenidate and rats trained to discriminate methylphenidate were tested with triadimefon. Triadimefon substituted completely for methlyphenidate and methlyphenidate substituted completely for triadimefon. These results indicate that triadimefon can function as a discriminative stimulus and that it shares discriminative stimulus properties with methylphenidate.

Triadimefon Methylphenidate Drug discrimination Rats

THE behavioral effects of a variety of pesticides have been evaluated using locomotor activity and schedule-controlled behavior. Typically, pesticides decrease overall response rates of schedule-controlled behavior and levels of motor activity. A notable exception has been triadimefon, a pesticide that is used systemically on a number of cereals, fruits, and vegetables (7). Triadimefon appears to have some psychomotor stimulant-like properties; it has been found to increase levels of motor activity in rats (6,9) and to disrupt patterns of responding under fixed-interval schedules of reinforcement in a manner similar to the psychomotor stimulants methylphenidate and d-amphetamine (1,9). A number of investigators (5, 9, 14) have also reported triadimefon-induced stereotyped behaviors following large dosages that are similar to those produced by psychomotor stimulants.

The discriminative stimulus properties of drugs provide a useful means for assessing the class specificity of drugs (2). Psychomotor stimulants are capable of serving as discriminative stimuli and can control different behavioral responses in several species depending on whether they have received drug or saline. Once discriminative control has been established with a drug, other substances may then be substituted. Generally, closely related substances produce the response associated with the training drug, while compounds from other pharmacological classes produce the response associated with saline. Thus methylphenidate has been shown to substitute for cocaine in rats trained to discriminate cocaine from saline (16) and for d-amphetamine in rats trained to discriminate d-amphetamine from saline (8), but it does not substitute for pentylenetetrazol in rats trained to discriminate pentylenetetrazol from saline (12).

The present experiment was designed to assess the discriminative stimulus properties of triadimefon and to compare its effects with those of methylphenidate. A crossover design was used in which different rats were first trained with either triadimefon or methylphenidate and then tested with the other compound to assess the similarity of their discriminative stimulus characteristics.

METHOD

Animals

Eight experimentally naive male Long-Evans rats (Charles River, Raleigh, NC) weighing between 310–350 g were individually housed in standard ceiling-suspended stainless steel cages in a temperature-controlled room with a 12-hour light:dark cycle (lights on at 6:00 a.m). Rats received approximately 12 g of food (Purina Lab Blox) immediately following each session. Water was always available in the home cage.

Apparatus

Sessions were conducted in four standard operant chambers (Coulbourn Instruments Inc., Lehigh Valley, PA). Each cham-

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ber was housed in a light- and sound-attenuating ventilated enclosure. On one wall of the chamber, a house light was mounted centrally above a recess in which milk (one part Eagle Brand sweetened condensed milk to two parts tap water) could be presented by a dipper mechanism. The houselight was illuminated throughout the session, except during reinforcement. Reinforcement consisted of 4-s access to approximately 0.05 ml of milk. On either side of the recess were two response levers. Scheduling of reinforcement contingencies and data collection were performed through computer control (15) allowing 0.01 s precision of timing.

Procedure

Lever pressing was first established by shaping and ultimately reinforced according to a tandem variable interval 1-min fixed ratio-10 (Tand VI 1-min FR-10) schedule. The tandem schedule was chosen because the irregular intervals separating reinforcement make performance relatively insensitive to change during extinction sessions (13). Thus, during extinction test sessions, the tandem schedule maintains relatively higher rates of responding as compared to an FR-10 schedule (13).

Pressing was established for one lever and was then established for the other. Only one lever was available during the initial phase of training; the lever that was not in use was covered by a metal box that prevented access to it. The subjects were initially trained to lever press according to an FR-1 schedule of reinforcement. Once responding was established, experimental sessions were arranged five days per week. The value of the fixed ratio was gradually increased to FR-10. After approximately five sessions under the FR-10 schedule, an interval requirement was introduced such that 10 responses were required after 30 s had elapsed for reinforcement (Tand FI-30 s FR-10). Following two sessions under this schedule, the terminal schedule was introduced such that the 10th lever press was reinforced after a variable interval of 1 min (range: 2.4–229.0 seconds) had elapsed (Tand VI 1-min FR-10). The intervals were determined using a constant-probability formula described by Catania and Reynolds (4). The order of the intervals was arranged such that every sequence of five intervals totalled approximately 300 s, thereby ensuring that the rats did not receive a series of long or short interreinforcement intervals. Additionally, five different series of intervals were used so that the rats were not exposed to the same series every day. Sessions ended either after 25 reinforcements or 25 minutes had elapsed, whichever occurred first.

Once the baseline of responding had been established, both levers were made available simultaneously and drug injections were begun. In order to minimize effects due to position preferences, the subjects were divided into two groups. For one group, responding on the left lever was reinforced whenever drug was injected, whereas for the other group responding was reinforced on the right lever after drug injections. Responses on the opposite levers were reinforced after saline injections. Additionally, a reset contingency was added to prevent superstitious chaining of responses between the levers. Under this arrangement, ten consecutive responses on the correct lever were required for reinforcement, while each incorrect response reset the FR requirement. Incorrect responses during the VI component of the tandem schedule were recorded but had no other programmed consequences.

Four rats were trained to discriminate injections of saline from injections of methylphenidate (4.0 mg/kg, IP, 20 min before a session) and four rats were trained to discriminate injections of saline from injections of triadimefon (40 mg/kg, IP, 30 min before a session). Saline, not triadimefon vehicle, was used as the nondrug training condition. Drug and saline injections alternated nonsystematically during training, with the restriction that the same solution could not be given for more than 3 successive sessions, so that each condition was in effect for approximately an equal number of sessions. Initial sessions with triadimefon used a 30 mg/kg training dose; when this dose proved to be ineffective as a discriminative stimulus (about 39 sessions), the dose was increased to 40 mg/kg. When the 40 mg/kg dose also failed to produce discriminative stimulus control of responding after 11 sessions, the dose was increased to 80 mg/kg, which did engender stimulus control within 1 to 8 sessions. However, after 78 sessions using 80 mg/kg as the training dose, one of the rat's response rates decreased considerably, presumably due to interfering stereotyped behaviors. The training dose was then reduced to 40 mg/kg for all rats. All data presented for the triadimefon-trained rats represent responses made after the training dose was reduced to 40 mg/kg.

Responses emitted before the first reinforcement were used to determine the degree of discriminative control in training sessions. Discriminative control was defined as ten successive sessions of greater than 80% correct lever selections prior to the first reinforcement following administration of either drug or saline. Once this criterion was met, generalization tests occurred (see below) whenever the following criteria were met for two consecutive sessions: responding on the appropriate lever prior to the first reinforcement was greater than 80% and greater than 75% responding to the appropriate lever was maintained for the entire session.

Generalization Testing

At the conclusion of the acquisition phase, rats were tested with various doses of the training drug in order to determine a dose-response function. The rats trained to discriminate methylphenidate from saline were tested with saline and several additional doses of methylphenidate (0.5, 1, 2, 4 and 8 mg/kg). The rats trained to discriminate triadimefon from saline were tested with the triadimefon vehicle (5% ethanol, 5% Emulphor and 90% saline) and several additional doses of triadimefon (10, 20 and 40 mg/kg). Doses were administered in a nonsystematic order. Tests were given on Tuesdays and Fridays as 5-min extinction sessions (no reinforcers given). Training conditions were in effect on the other three days of the week in order to maintain stimulus control (criterion sessions, see above).

Cross-generalization testing began after the initial dose-response curves were determined and utilized the same pattern of test and criterion sessions. Rats trained to discriminate methylphenidate from saline were tested with triadimefon (10, 20, 40, 60, 80 and 100 mg/kg, injected 30 min before a session) and the triadimefon vehicle, while rats trained to discriminate triadimefon from saline were tested with methylphenidate (0.5, 1, 2, 4 and 8 mg/kg, injected 20 min before a session) and saline.

Data Analysis

The data are expressed as the mean (±SEM) percentage of responses made on the drug-appropriate lever per trial. Response rate data are shown as the mean (±SEM) response rate and were calculated by dividing the total number of responses emitted on either lever by the session duration. Compounds were considered to share discriminative stimulus effects for a rat if they occasioned at least 80% drug-appropriate responding.

Drugs

Methylphenidate hydrochloride (donated by Ciba-Geigy, Summit, NJ) was dissolved in physiological saline. Triadimefon

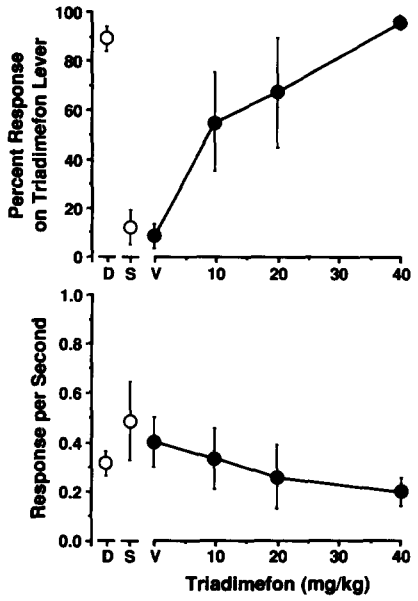

FIG. 1. Effects of triadimefon in rats trained to discriminate triadimefon from saline. Top panel: Ordinate: mean percentage ± 1 SEM, of responses made on the drug lever during test sessions. Lower panel: Ordinate: mean response rate on both levers ± 1 SEM, expressed as responses per second. The abscissae indicate the dose of triadimefon in milligrams per kilogram body weight. D = mean responding from drug criterion sessions; S = mean responding from saline criterion sessions. Each data point is based on n = 4.

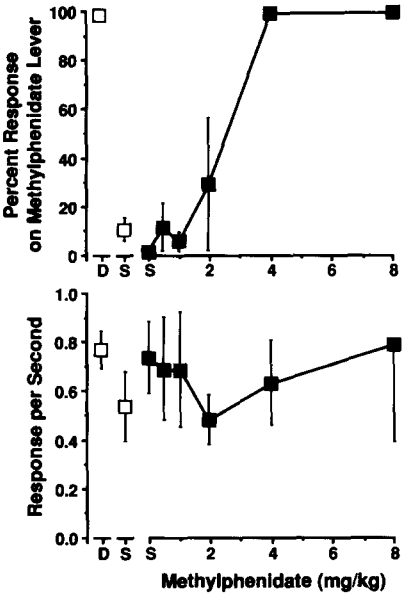

FIG. 2. Effects of methylphenidate in rats trained to discriminate methylphenidate from saline. Top panel: Ordinate: mean percentage ± 1 SEM, of responses made on the drug lever during test sessions. Lower panel: Ordinate: mean response rate on both levers ± 1 SEM, expressed as responses per second. The abscissae indicate the dose of methylphenidate in milligrams per kilogram body weight. The S on the unbroken portion of the abscissa represents mean responding on the drug lever after saline injections. D = mean responding from drug criterion sessions; S = mean responding from saline criterion sessions. Each data point is based on n = 4.

(purchased from Chem Service, West Chester, PA) was suspended in a vehicle of 5% Emulphor, 5% ethanol and 90% physiological saline. All compounds were injected IP in a volume of 1 ml/kg body weight.

RESULTS

Generalization

During criterion sessions, triadimefon occasioned 89 ± 4% triadimefon-appropriate responses, whereas saline produced 13 ± 6% triadimefon-appropriate responses (Fig. 1). The mean response rate during criterion sessions following triadimefon administration was approximately 70% of saline responding. Triadimefon occasioned responding on the drug-appropriate lever in a dose-related manner, with complete generalization obtained at 40 mg/kg. The variability in the percent drug lever responding at intermediate doses reflects the responding of one subject that selected the saline-appropriate lever at both 10 and 20 mg/kg triadimefon. Mean responding on the drug-appropriate lever after injections of triadimefon vehicle during test sessions did not differ from that after injections of saline during criterion sessions. These results indicate that the discriminative control exerted by triadimefon vehicle and saline were indistinguishable. Response rates obtained during test sessions were within the range of the mean rates obtained during triadimefon and saline criterion sessions. There was no systematic relationship between response rate and percent correct choice on the levers.

Methylphenidate occasioned 98 ± 0.4% methylphenidate-appropriate responses during criterion sessions (Fig. 2). Following saline administration, 11 ± 4% methylphenidate-appropriate responses were produced during criterion sessions. Methylphenidate occasioned dose-dependent responding on the drug-appropriate lever; complete generalization occurred at both 4 and 8 mg/kg methylphenidate. The variability in the percent drug lever responding at 2 mg/kg methylphenidate reflects the responding of one subject that generalized responding to the drug-appropriate lever. Response rates obtained during some methylphenidate test sessions were slightly elevated relative to the mean rate of responding during saline criterion sessions, but were within the range of the mean rate of drug responding during methylphenidate criterion sessions. There was no systematic relationship between percent correct choice on the levers and rate of responding.

Cross-Generalization

For the rats trained to discriminate triadimefon, methylphenidate (0.5–8 mg/kg) produced a dose-related increase in responding on the drug-appropriate lever (Fig. 3). Doses of 2, 4 and 8 mg/kg methylphenidate substituted completely for triadimefon. Saline exerted a similar degree of stimulus control of responding during both the cross-generalization test sessions (Fig. 3) and criterion sessions (Fig. 1). Although response rates for the triadimefon-trained rats were suppressed during the cross-generalization phase of the experiment, the decreases in response rates were unrelated to the dose of methylphenidate and to the accuracy of responding.

Similarly, triadimefon (60–100 mg/kg) produced drug-appropriate responding in the rats trained to discriminate methylphenidate. Triadimefon substituted completely for methylphenidate following administration of 60, 80 and 100 mg/kg. Percent responding on the drug lever following triadimefon vehicle was

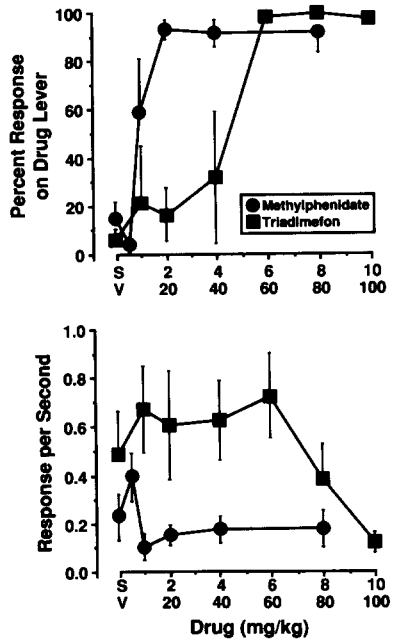

FIG. 3. Effects of methylphenidate in rats trained to discriminate triadimefon from saline (circles) and of triadimefon in rats trained to discriminate methylphenidate from saline (squares). Top panel: Ordinate: mean percentage ± 1 SEM, of responses made on the drug lever during test sessions. Lower panel: Ordinate: mean response rate on both levers ± 1 SEM, expressed as responses per second. The abscissae indicate the doses in milligrams per kilogram body weight: the upper scale represents the dose of methylphenidate and the lower scale the dose of triadimefon. Each data point is based on n = 4.

comparable to that obtained following saline during generalization test sessions. Mean rate of responding on the drug-appropriate lever following injection of either triadimefon vehicle or low doses of triadimefon (Fig. 3) was similar to the mean rate of responding after injection of either saline or low doses of methylphenidate (Fig. 2). Higher doses of triadimefon markedly suppressed response rates, although at these doses the rats continued to choose the drug-appropriate lever.

DISCUSSION

The discriminative stimulus properties of the pesticide triadimefon were qualitatively similar to those produced by the psychomotor stimulant methlyphenidate. For rats trained to discriminate a dose of 40 mg/kg triadimefon from saline, methylphenidate substituted completely for the triadimefon stimulus. Further, for rats trained to discriminate a dose of 4 mg/kg methylphenidate from saline, triadimefon substituted completely for the methylphenidate stimulus. Complete cross-generalization occurs between methlyphenidate and other psychomotor stimulants such as cocaine and d-amphetamine regardless of which drug is used in training (8). It therefore seems likely that triadimefon would substitute for these stimulants as well.

The finding that methylphenidate and triadimefon are cross-generalized raises implications regarding the potential abuse liability of triadimefon. Drug discrimination data are frequently used as indirect indices of abuse liability (3, 10, 11). The utility of drug discrimination is that it allows one to assess the extent to which the discriminative stimulus effects of a novel drug are similar to the discriminative stimulus effects of a reference drug of known abuse liability, i.e., methylphenidate (11). Thus it is possible to predict whether the abuse potential of a drug is of a particular pharmacological class, for example whether it is of the "psychomotor stimulant-type." Given triadimefon's commercial availability and the similarity of its effects to the psychomotor stimulants, it is clear that more work needs to be done in order to determine if triadimefon has abuse potential.

Taken together, the current data for triadimefon suggest that its actions in the CNS are similar to those of the psychomotor stimulants, e.g., methylphenidate (5, 9, 14). However, further studies are necessary before a comparison between triadimefon and the psychomotor stimulants may be stated conclusively. It would be interesting to confirm that the discriminative stimulus produced by triadimefon is specific to the psychomotor stimulants; i.e., it would not generalize to drugs from other pharmacological classes. Further, it would be interesting to determine if triadimefon's neuronal mechanism of action involves the dopaminergic system.

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