Pharmacology Biochemistry & Behavior, Vol. 35, pp. 971–980. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Three-Choice Discrimination Among (+)-Amphetamine, Fenfluramine and Saline in Pigeons[1]

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Received 22 May 1989

EVANS, S. M., J. P. ZACNY AND C. E. JOHANSON. *Three-choice discrimination among (+)-amphetamine, fenfluramine and saline in pigeons.* PHARMACOL BIOCHEM BEHAV **35**(4) 971–980, 1990.—Five pigeons were trained to discriminate among (+)-amphetamine (AMPH; 1.7 or 3.0 mg/kg), fenfluramine (FEN; 5.6 or 10 mg/kg), and saline using a three-choice drug discrimination procedure. The results of the study demonstrated that a reliable discrimination between AMPH and FEN could be obtained and the discriminative stimulus (DS) effects of these two drugs did not overlap, i.e., were mutually exclusive. Phenmetrazine produced a dose-related increase in AMPH-appropriate responding with no responding occurring on the FEN-appropriate key. Two serotonin agonists, quipazine (5-HT_2) and MK 212 (5-HT_1), produced FEN-appropriate responding in two of three pigeons, while a third pigeon responded predominantly on the AMPH-appropriate key following their administration. In contrast, phencyclidine produced predominantly (greater than 50%) saline-appropriate responding, indicating that the DS effect of phencyclidine was unlike either AMPH or FEN. Finally, compounds known to have multiple DS properties such as MDA and MDMA were tested. The results with these compounds confirmed that these drugs have complex DS effects both within and across individual pigeons.

Drug discrimination Pigeons (+)-Amphetamine Fenfluramine MDA MDMA

AMPHETAMINE (AMPH) and fenfluramine (FEN) are similar structurally and both are used therapeutically as anorectics. However, they have different profiles of neurochemical and behavioral effects. Neurochemically, the effects of AMPH are mediated predominantly by the release of catecholamines (CA), particularly dopamine (DA) (14,25); in contrast, the effects of FEN are mediated by the release of serotonin (5-HT) (8,21). Although both compounds decrease food intake there are differences in their behavioral effects in other paradigms. For instance, AMPH is self-administered by rhesus monkeys (1) and humans (22), whereas FEN is not self-administered by either species (23,41). In addition, AMPH increases locomotor activity in rats (4) and produces stimulant-like subjective effects in humans (3); FEN has sedative properties in rats (42) and has a profile of subjective effects in humans that is not stimulant-like (3, 15, 19).

In two-choice drug discrimination studies, results with these compounds have been inconsistent. In some studies, AMPH and FEN have been found to be dissimilar (9, 27, 31, 34), while other studies have shown an overlap in the DS properties of AMPH and FEN (3, 7, 16). For instance, in rats trained to discriminate 3.0 mg/kg FEN from saline, 0.25 mg/kg AMPH substituted for FEN in 3 of 7 rats (16). In a previous study using pigeons, FEN substituted for AMPH in 2 of 4 pigeons, and produced partial substitution in another pigeon (7). In addition, in humans trained to discriminate AMPH from placebo, 40 mg FEN produced AMPH-appropriate responding as well as AMPH-like subjective effects in 50% of the participants (3). Thus, it appears that AMPH and FEN have some effects in common but are not identical as DS.

Three-choice drug discrimination procedures have been used with drugs that could not be differentiated when two-choice drug discrimination procedures were used. For instance, in two-choice drug discrimination studies, partial substitution was observed between the mixed opiate agonist-antagonist cyclazocine and the opiate agonist morphine in rats trained to discriminate either cyclazocine or morphine from saline (32,33). On the other hand, in animals trained in a three-choice drug discrimination procedure between cyclazocine, morphine and saline, there was no partial substitution and animals responded exclusively on the drug-appropriate lever across a wide range of doses of both compounds. Furthermore, the mixed agonist-antagonist levallorphan, which

[1]Supported by the National Institute on Drug Abuse Grant DA 00250 (C. R. Schuster, Principal Investigator). Portions of these data were presented to the Society on Stimulus Properties of Drugs in June, 1986.

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partially substituted for both morphine and cyclazocine in rats trained to discriminate either of these drugs from saline (32,33), produced exclusively cyclazocine-appropriate responding in the three-choice procedure with no indication of morphine-like effects (38). Thus, the use of a three-choice drug discrimination procedure appears to have resulted in a more selective discrimination which may be advantageous when compounds with multiple actions are evaluated.

The present study was designed to utilize a three-choice drug discrimination procedure in pigeons to differentiate between the DS properties of AMPH and FEN which have not been clearly differentiated under two-choice conditions. The first goal was to establish a reliable discrimination among AMPH, FEN and saline. Once established, drugs previously shown to substitute for either AMPH or FEN in standard two-choice drug discrimination paradigms were evaluated with the expectation that similar results would be generated in a three-choice drug discrimination paradigm, thus confirming the reliability of the paradigm. To further evaluate the pharmacological specificity of the discrimination, phencyclidine (PCP), a drug pharmacologically unrelated to either AMPH or FEN, was also tested.

Another purpose of training a three-choice discrimination was to test compounds that may affect multiple CNS systems. In two-choice drug discrimination paradigms, (±)-3,4-methylenedioxyamphetamine (MDA) has been shown to have both stimulant-like and hallucinogen-like DS properties (11,20). In particular, MDA has been shown to substitute for AMPH in rhesus monkeys (24), rats (11) and pigeons (6). However, MDA also has been shown to substitute for the hallucinogen, DOM (12) and recent data suggest that the DS effects of DOM are mediated by serotonin at 5-HT_2 receptors (10). The isomers of MDA have been shown in two-choice drug discrimination studies to have different profiles of activity, i.e., (−)-MDA substituted for the hallucinogen DOM and (+)-MDA did not (13). In contrast, (+)-MDA substituted for AMPH but (−)-MDA did not (11).

The structural analog of MDA, (±)-3,4-methylenedioxymethamphetamine (MDMA), is reported to produce more stimulant-like properties than MDA. As with MDA, in two-choice drug discrimination studies MDMA produced AMPH-like responding (6, 11, 24) and also substituted for drug in animals trained using MDA (11), but failed to substitute for DOM (13). However, other studies have demonstrated that MDMA has multiple actions. MDMA substituted completely for the indirect DA agonist (−)-cathinone and also substituted for two serotonergic compounds, namely, FEN and tetrahydro-β-carboline (29). Furthermore, there is evidence that MDMA binds to 5-HT receptors with an affinity similar to that of MDA (26). Taken together, the results of these studies suggest that both MDA and MDMA have dopaminergic as well as serotonergic properties.

In summary, the present study was designed to establish a discrimination between two anorectics (AMPH and FEN) that share DS properties to a partial extent. In addition, this experiment was designed to determine whether the acquired discrimination was at least as selective as respective two-choice drug discrimination procedures. This was assessed by testing drugs such as phenmetrazine, quipazine, MK 212 and PCP that have been shown to substitute for AMPH, FEN or saline in two-choice drug discrimination studies. Finally, compounds that affect multiple CNS systems (e.g., MDA and MDMA) were tested in order to determine whether this procedure can be used to evaluate the relative contribution of different neurochemical mechanisms to the DS properties of mixed action drugs.

METHOD

Animals

The animals used in this study were five female White Carneaux pigeons. At the beginning of the experiment the pigeons were 3 years old and experimentally naive. The pigeons were maintained at 80% of their free-feeding weights and housed individually with water and grit freely available. Purina Pigeon Checkers were provided after the session to maintain reduced weights.

Apparatus

The experiment was conducted in one ventilated custom-made operant chamber (inside dimensions 32 × 28 × 32 cm). The front and back panels were aluminum and the side walls were transparent plastic. The chamber was equipped with three translucent response keys (2.5 cm diameter; G6315, Ralph Gerbrands Co., Arlington, MA) which were transilluminated during the experimental session by white 7-W lamps (IEE, Van Nuys, CA) located behind the keys. The keys were 5 cm apart and located approximately 24 cm above the floor of the chamber. Purina Pigeon Checkers were made available from a food magazine (G5610A, Ralph Gerbrands Co., Arlington, MA) centered between and below the response keys, 7.6 cm above the floor. This food magazine was illuminated during food delivery. A 6-W white houselight, located behind the back panel, provided indirect illumination during experimental sessions. Programming and data collection were accomplished using a Rockwell AIM 65 microcomputer and cumulative recorders (Ralph Gerbrands Co., Arlington, MA) which were located in an adjacent room.

Training

Initial key peck training consisted of shaping the response until it met the requirements of a fixed-ratio 30 (FR 30) for food reinforcement on all three keys. This was accomplished by making only one key available during a session while the other two were covered; the operative key changed daily. Once responding under the FR schedule was established on the 3 keys, IM injections of AMPH, FEN or saline were administered in a 1.0 ml/kg volume 10 min before the session in the following sequence (AMPH, FEN, SAL, SAL, AMPH, FEN, FEN, SAL, AMPH, AMPH, SAL, FEN, etc.). Following the 10-min pretreatment period, the experimental session began, signalled by the illumination of the injection-appropriate key and the houselight. Thirty consecutive responses on the injection-appropriate key resulted in 3-sec access to food. Each session lasted until 50 reinforcers had been delivered or until 30 minutes had elapsed, whichever occurred first.

In order to associate each drug condition with a particular key, each drug condition initially was presented with only one key illuminated and the other two keys covered. When all training stimuli had been presented under these conditions for several sessions, two keys were then available during each session but a reinforcer was delivered only for responding on the injection-appropriate key. The second (incorrect) key alternated such that for each AMPH training session, the saline key was the alternative 50% of the time and the FEN key the other 50%. The same alternate presentation of keys was instituted for saline and FEN training sessions. Responses on the incorrect key reset the FR requirement on the correct key. Once responding came under stimulus control under all 6 paired conditions, all three keys were illuminated simultaneously, again with only the injection-appropriate key associated with reinforcement delivery. This training procedure was modified slightly for pigeons 3125 and 3492. For these two pigeons, training was as described except that once all training stimuli had been presented for 30 to 45 sessions with only the injection-appropriate key available, then all three keys became available simultaneously. Thus, the phase of uncovering only two keys at a time was eliminated while the duration of the phase of

having only the injection-appropriate key available was increased. For all pigeons, the discrimination was established by presenting the same number of sessions with each stimulus condition in an effort to minimize the development of a key bias. The terminal training doses were 1.7 mg/kg AMPH and 5.6 mg/kg FEN for pigeons 4304 and 4704 and 3.0 mg/kg AMPH and 10 mg/kg FEN for pigeons 7216, 3125, and 3492. However, considerable training had been attempted with other doses of AMPH (1.0–3.0 mg/kg) and FEN (3.0–10 mg/kg) with 3 of the 5 pigeons (4304, 4704, and 7216). Training with the terminal training doses continued until the percent of total responses during a session on the injection-appropriate key was above 90%. In addition, the number of responses emitted on the two incorrect keys was required to be less than 30 before the first reinforcer was received. These criteria had to be met for 7 out of 9 consecutive sessions and a further restriction was that the two noncriterion sessions not be under the same drug condition.

Testing

In order to assess the DS properties of other doses of the two training drugs, as well as evaluate additional compounds, test sessions were conducted. Throughout a test session, 30 consecutive responses on the AMPH-, FEN-, or saline-appropriate key resulted in food delivery. In all other respects, test sessions were identical to training sessions. Test sessions were scheduled to occur every fourth session (i.e., . . . AMPH, FEN, SAL, TEST, SAL . . .) as long as responding during the three preceding training sessions (one under each condition) also met criteria. If an animal failed to meet the training criteria during a training session, the test was postponed until the animal met the 3-session criterion.

Initially, dose-response functions for AMPH and FEN were established during test sessions. Subsequently, the effects of other drugs were determined in a similar manner. Each dose of a test compound was tested once and at least three doses, ranging from a dose that produced predominantly saline-appropriate responding to one that reduced response rate, were tested in a mixed order. For each pigeon, the dose-response function for each drug was completed before another compound was tested. After the completion of each dose-response function, pigeons were given test sessions with one of the training drugs (AMPH, FEN, or SAL).

Data Analysis

The discrimination data are presented as the percentage of total responses made on each of the drug keys (AMPH and FEN) for individual pigeons. The percentage of saline-appropriate responding is not shown, but is equal to the difference between 100% and the sum of AMPH- and FEN-appropriate responding. If no reinforcers were obtained during the test session, the data were not included in the analysis of drug-appropriate responding. In addition to recording the distribution of responses, response rate (responses/sec) on all three keys was determined for each session.

A test drug was considered to produce DS effects similar to those of AMPH if at least 80% of the total responses during the test session were emitted on the AMPH-appropriate key. Likewise, a drug was considered to produce DS effects similar to those of FEN if at least 80% of the total responses were emitted on the FEN-appropriate key. Even if responding was greater than 80% on either drug key, testing of higher doses in subsequent sessions continued until response rate was substantially reduced (to at least 50% of the rate for the training drugs) since for some compounds the DS properties might shift as a function of dose.

Drugs

The following drugs used in this experiment were gifts: (+)-amphetamine sulfate, (±)-MDA HCl, (+)-MDA HCl, (−)-MDA HCl, (±)-MDMA HCl, and phencyclidine HCl (National Institute on Drug Abuse), fenfluramine HCl (A. H. Robins Co., Richmond, VA), MK 212 HCl (Merck Sharp and Dohme, West Point, PA), quipazine maleate (Miles Laboratories, Inc., Elkhart, IN) and phenmetrazine HCl (Boehringer Ingelheim, LTD., Ridgefield, CT). All drugs were dissolved in physiological saline and the doses of these drugs are expressed in terms of the salt.

RESULTS

Control Performances

Originally, training was attempted with 1.0 mg/kg AMPH and 3.0 mg/kg FEN for pigeons 4304, 7216 and 4704. However, since even after several months the discrimination was not acquired, the training doses of AMPH and FEN were increased to 3.0 and 10 mg/kg, respectively. For two pigeons (4304 and 4704), these doses suppressed responding; they were then trained at 1.7 mg/kg AMPH and 5.6 mg/kg FEN, the dosing regimens at which they acquired the discrimination. Pigeon 7216 continued to be trained to discriminate 3.0 mg/kg AMPH from 10 mg/kg FEN from saline, and eventually acquired the discrimination. The other two pigeons (3125 and 3492) were initially trained to discriminate 3.0 mg/kg AMPH from 10 mg/kg FEN from saline, and no dose changes were required. Thus, for pigeons 4304 and 4704 the terminal training doses were 1.7 mg/kg AMPH and 5.6 mg/kg FEN, whereas 3.0 mg/kg AMPH and 10 mg/kg FEN were the terminal training doses for pigeons 7216, 3125 and 3492. Sessions to criteria, determined from the first day of the terminal training dose regimen with all three keys available, ranged between 32 and 157 sessions (mean = 83 sessions). Pigeons 3125 and 3492, that were trained with the higher doses of AMPH and FEN and required no modification in the training procedure, acquired the discrimination in 32 and 39 days, respectively. Pigeon 4304 failed to maintain DS control after the dose-response functions for AMPH and FEN were completed and was removed from the study. Pigeon 4704 died of a drug overdose near the end of the study and pigeon 3492 was added as a replacement.

Table 1 shows the results of test sessions with the training drugs and saline for each pigeon throughout the study, the number of sessions to criteria and the final doses used for training each pigeon. During test sessions when saline was administered, no more than an average of 5% of the total responses were made on either the AMPH- or FEN-appropriate key for any pigeon. The training dose of AMPH (1.7 or 3.0 mg/kg) administered during test sessions reduced response rates from 65% to 73% of the saline rate. The training dose of FEN (5.6 or 10 mg/kg) administered during test sessions reduced response rates similar to the rate reduction observed with the training dose of AMPH. In summary, saline rates were higher than either FEN or AMPH rates and the rates following AMPH and FEN were similar for all pigeons.

The dose-response functions for AMPH are shown in Fig. 1 (top panels) as the percent of total responses on each of the two drug keys for individual pigeons. AMPH (0.3–5.6 mg/kg) produced a dose-related increase in the percentage of responses emitted on the AMPH-appropriate key. At any dose of AMPH tested, less than 3% of the total responses were made on the FEN-appropriate key by any pigeon. Four of five pigeons responded predominantly on the AMPH-appropriate key only at their respective training dose or a higher dose, i.e., low doses of AMPH produced predominantly saline-appropriate responding. AMPH also produced a dose-related decrease in response rate (Fig. 1, lower panels).

FEN (1.0–17 mg/kg) produced a dose-related increase in the percentage of responses emitted on the FEN-appropriate key (Fig. 2, upper panels). As with AMPH, at all doses of FEN tested,

TABLE 1

TRAINING DOSE TEST SESSIONS FOR INDIVIDUAL PIGEONS

Pigeon	STC*	Drug Dose (mg/kg; IM)	N†	Test Sessions with Training Drugs‡ %FEN	%SAL	%AMPH	Rate§
4304	157	FEN 5.6	1	98	1	1	1.46
		SAL	1	0	100	0	2.29
		AMPH 1.7	1	0	0	100	1.32
4704	115	FEN 5.6	3	98 (1)	2 (1)	0	1.44 (0.09)
		SAL	4	1 (1)	94 (6)	5 (5)	1.95 (0.13)
		AMPH 1.7	4	0	0	100	1.58 (0.17)
3125	32	FEN 10.0	3	100	0	0	1.71 (0.31)
		SAL	3	0	100	0	2.74 (0.02)
		AMPH 3.0	3	0	0	100	1.60 (0.18)
3492	39	FEN 10.0	1	99	0	1	1.30
		SAL	3	1	98 (1)	1	2.31 (0.03)
		AMPH 3.0	2	0	0	100	1.31 (0.03)
7216	72	FEN 10.0	4	99 (0.7)	0	1 (0.9)	1.85 (0.11)
		SAL	4	0	100	0	2.69 (0.11)
		AMPH 3.0	4	1 (0.6)	0	99 (0.6)	1.78 (0.07)

*STC = Sessions to criterion for acquiring the discrimination.
†Number of test sessions with the training drugs throughout the study.
‡Mean percentage of responses on the three respective keys (± 1 S.E.M.).
§Response rate is expressed as responses/second.

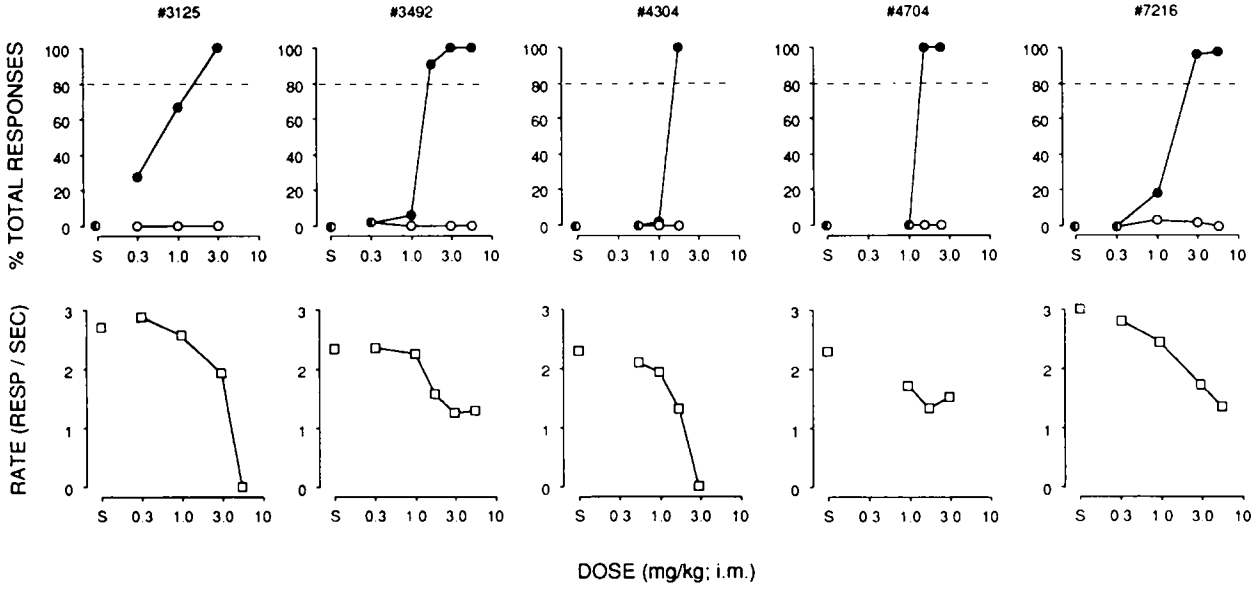

FIG. 1. The dose-response functions for amphetamine (AMPH) for individual pigeons. The ordinate for the top panels represents the percentage of responses made on each of the drug keys. The amount of saline-appropriate responding is not shown but is equal to the difference between 100% and the sum of AMPH- and FEN-appropriate responding. The points above S are the percentage of AMPH- and FEN-appropriate responding following saline test sessions. The dashed line represents the 80% criterion used to determine if a drug dose substituted for AMPH or FEN. The lower panels represent response rates for individual pigeons. Two pigeons (4304 and 4704) were trained to discriminate among 1.7 mg/kg AMPH, 5.6 mg/kg FEN and saline, while the other pigeons were trained to discriminate among 3.0 mg/kg AMPH, 10 mg/kg FEN and saline. ○: FEN; ●: AMPH; □: RATE.

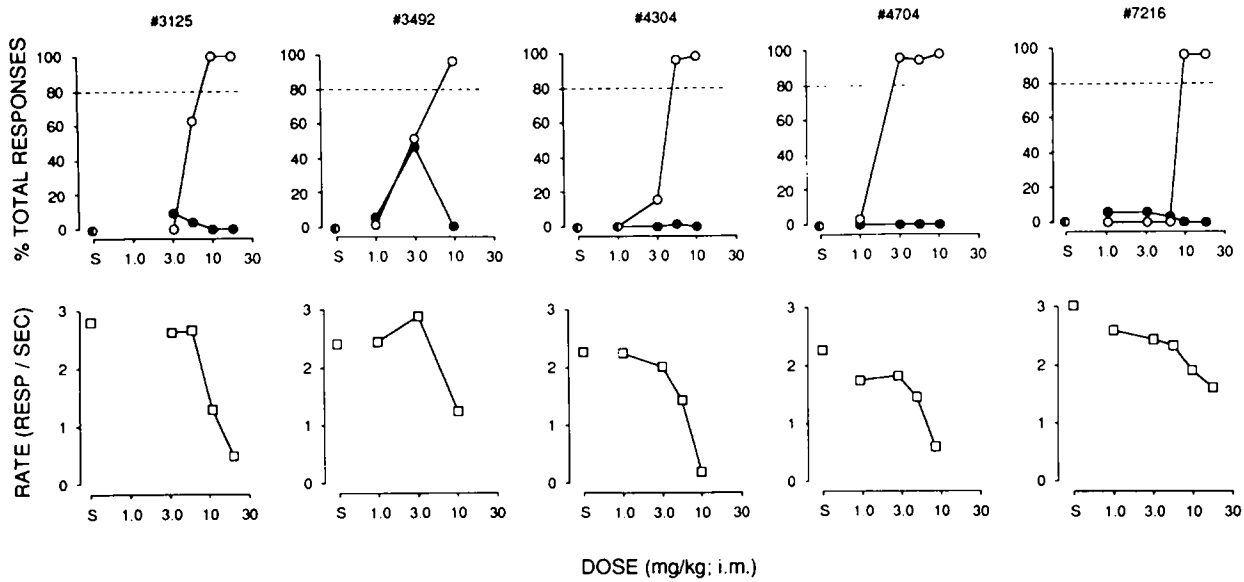

FIG. 2. The dose-response functions for fenfluramine (FEN) for individual pigeons. Other details as described for Fig. 1.

responding on the AMPH-appropriate key was low (less than 9%) with the exception of pigeon 3492 who responded 48% on the AMPH-appropriate key following 3.0 mg/kg FEN. In pigeon 4704, even doses of FEN lower than the training dose (5.6 mg/kg) produced predominantly FEN-appropriate responding. For the other pigeons the dose-response function of FEN was very steep; only the training dose of FEN produced greater than 80% FEN-appropriate responding. There was a dose-related decrease in response rate (Fig. 2, lower panels).

Phenmetrazine (1.0–30 mg/kg) produced a dose-related increase in AMPH-appropriate responding in all three pigeons tested (Fig. 3, upper panels). This drug did not produce any intermediate responding, i.e., responding occurred either exclusively on the AMPH-appropriate key or the saline-appropriate key at lower doses. Although there were differences in sensitivity across pigeons the doses of phenmetrazine that produced AMPH-appropriate responding were not correlated with the training dose. Phenmetrazine also produced a dose-related decrease in response rate (Fig. 3, lower panels).

Quipazine (0.1–5.6 mg/kg), a 5-HT_2 agonist, was also tested in four pigeons (Fig. 4). In two pigeons (3125 and 4704), quipazine produced a dose-related increase in responding on the FEN-appropriate key. However, there was a 10-fold difference in sensitivity between these two pigeons. In contrast, quipazine produced a dose-related increase in AMPH-appropriate responding in pigeon 7216 but at the dose (5.6 mg/kg) that produced over 80% AMPH-appropriate responding response rate was reduced to 0.15 responses/sec. In the fourth pigeon (3492), 3.0 mg/kg quipazine produced 50% AMPH-appropriate responding. The dose of 5.6 mg/kg completely suppressed responding so higher doses could not be tested. Thus, quipazine substituted reliably for FEN in 2 pigeons, for AMPH in a third pigeon, and in the fourth pigeon no consistent choice emerged. In all pigeons tested, quipazine produced a dose-related decrease in response rate (Fig. 4, lower panels).

The results from the 5-HT_1 agonist, MK 212 (0.3–5.6 mg/kg), are shown for individual pigeons in Fig. 5. MK 212 produced predominantly FEN-appropriate responding for pigeons 3125 and 7216. Responding on the AMPH-appropriate key did not exceed 36% at any dose tested for these two pigeons. In contrast, in pigeon 3492, MK 212 produced a dose-related increase in AMPH-appropriate responding which reached a maximum of 88% at 3.0 mg/kg. MK 212 produced a dose-related decrease in response rate. The testing of MK 212 could not be completed in pigeon 4704 since this pigeon died following the administration of 5.6 mg/kg MK 212. However, when 3.0 mg/kg MK 212 was tested on this pigeon, only 8% of the total responses were on the FEN-appropriate key, while 54% of the responses were on the AMPH-appropriate key and this dose did not decrease response rate. Therefore, as with quipazine, MK 212 substituted for FEN in 2 of 3 pigeons.

Figure 6 shows the results of testing PCP (0.1–1.7 mg/kg). PCP produced predominantly (greater than 50%) saline-appropriate responding at any dose tested in three pigeons. The highest doses tested completely suppressed responding (Fig. 6, lower panels).

The discrimination results with MDA, (+)-MDA, (−)-MDA and MDMA are shown in Fig. 7. There were no consistent trends across or within pigeons with any of the 4 compounds. Instead, pigeons tended to respond on both drug keys, the proportion of which varied with dose. Based on responding at the highest doses, MDA produced greater than 80% FEN-appropriate responding in 2 of 3 pigeons, whereas the third pigeon responded predominantly on the AMPH-appropriate key. As with MDA, (+)-MDA and (−)-MDA produced responding on both drug keys. At the highest doses that did not completely suppress responding, (+)-MDA produced greater than 80% FEN-appropriate responding in 2 of 3 pigeons and AMPH-responding in the third pigeon. In contrast, at the highest dose tested in each pigeon, (−)-MDA failed to produce greater than 70% responding on either the AMPH-appropriate or FEN-appropriate key. In contrast to MDA and (+)-MDA, MDMA produced greater than 80% AMPH-appropriate responding in 2 of 3 pigeons and the third pigeon responded predominantly on the FEN-appropriate key. Table 2 shows the response rate data for the drugs presented in Fig. 7. All of these drugs were tested up to doses that decreased response rate.

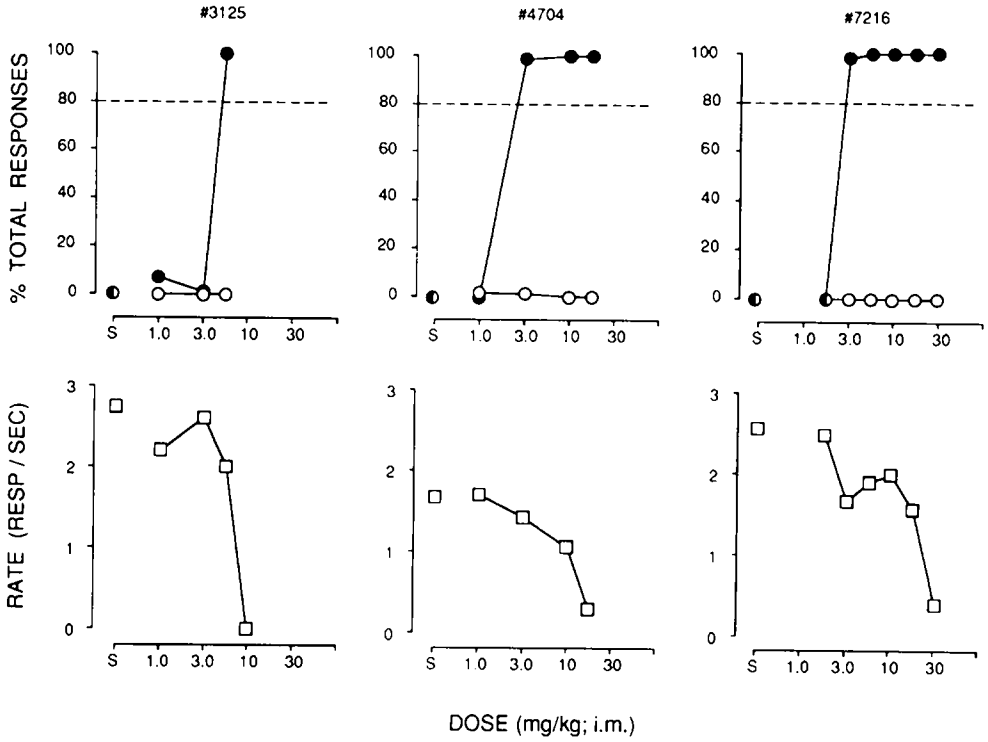

FIG. 3. The dose-response functions for phenmetrazine for individual pigeons. Other details as described for Fig. 1.

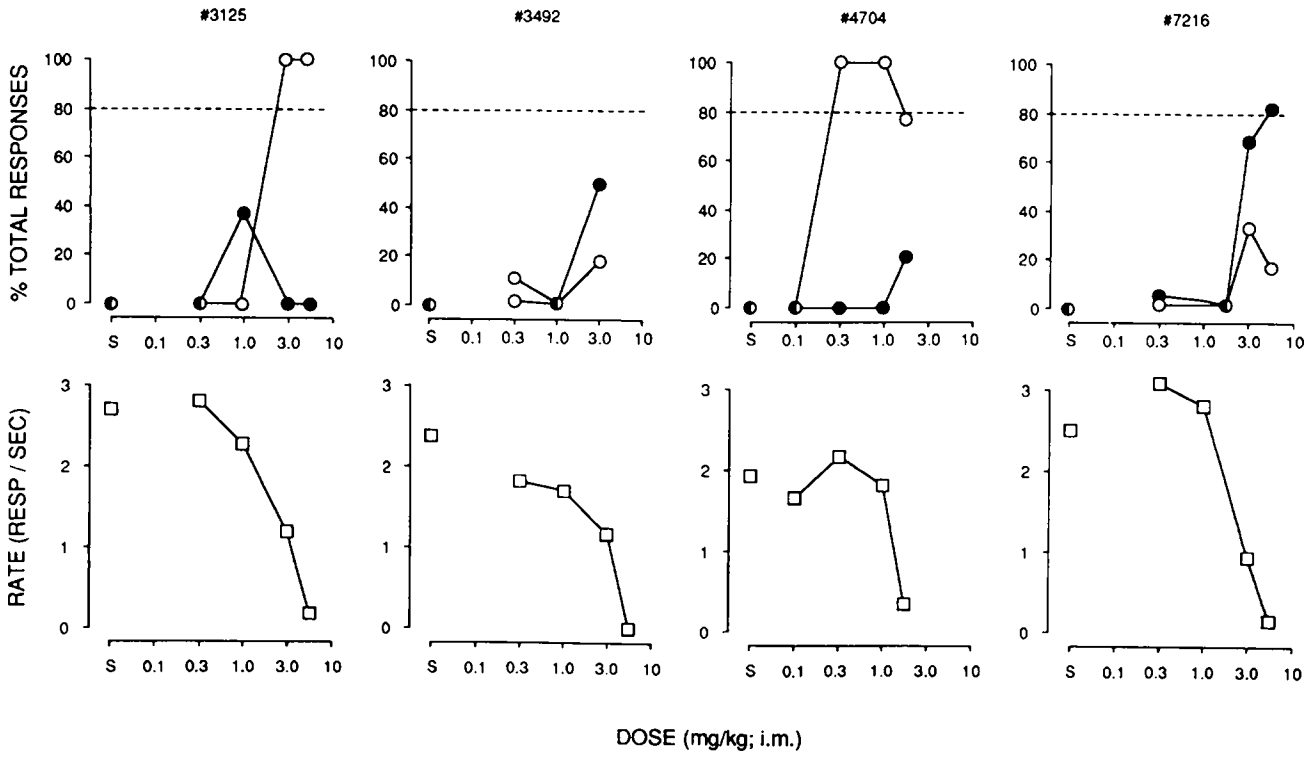

FIG. 4. The dose-response functions for quipazine for individual pigeons. Other details as described for Fig. 1.

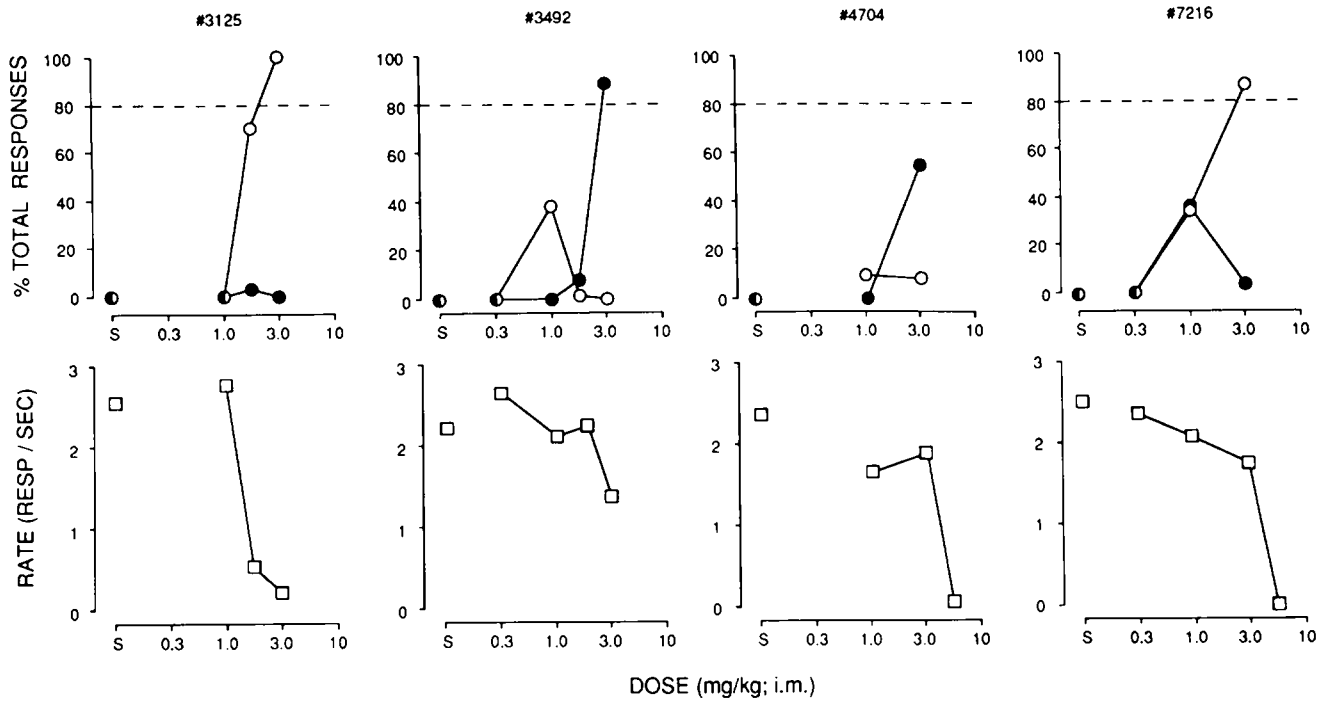

FIG. 5. The dose-response functions for MK 212 for individual pigeons. Other details as described for Fig. 1.

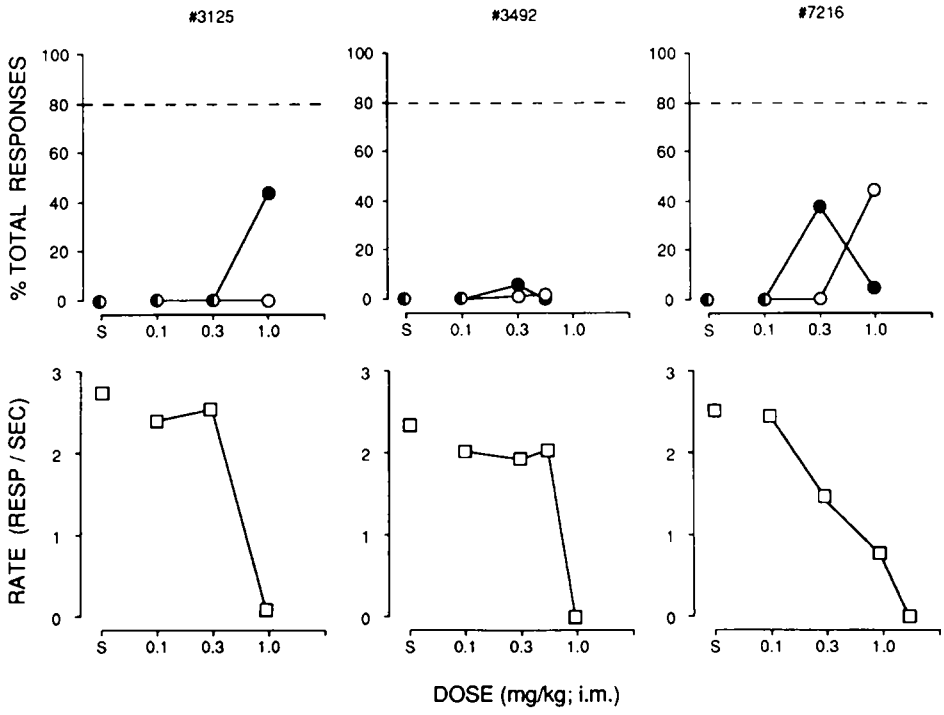

FIG. 6. The dose-response functions for phencyclidine for individual pigeons. Other details as described for Fig. 1.

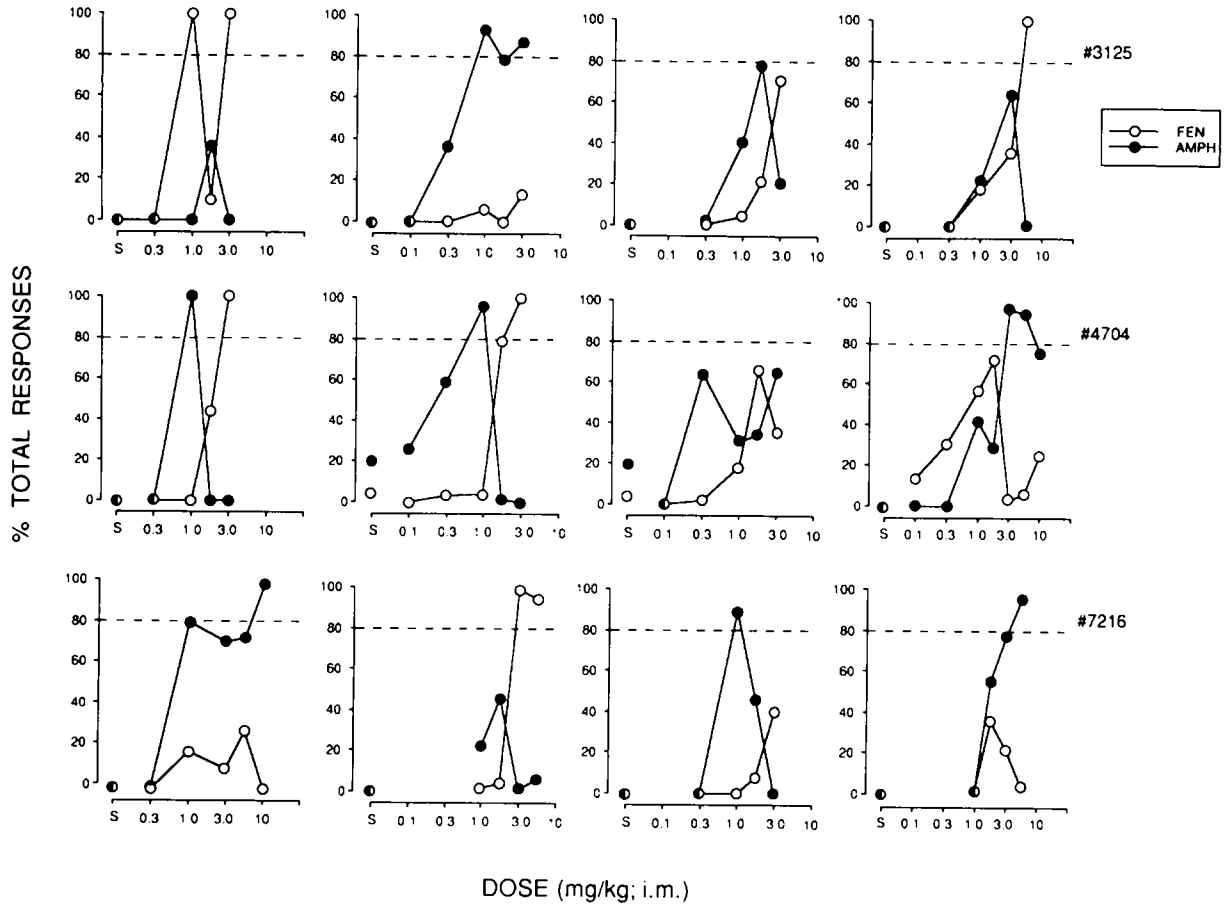

FIG. 7. The dose-response functions for (±)-MDA, (+)-MDA, (−)-MDA and (±)-MDMA for individual pigeons. Other details as described for Fig. 1.

DISCUSSION

This study demonstrates that a three-choice drug discrimination can be obtained among AMPH, FEN, and saline. Furthermore, there was no partial substitution between these two drugs, i.e., low doses of AMPH did not produce FEN-appropriate responding and the reverse was true for FEN. In addition, the training doses of AMPH and FEN reduced response rates to a similar degree. Taken together, these results indicate that the DS effects of these two drugs are mutually exclusive in a three-choice drug discrimination procedure.

In contrast to the results of the present study, results from studies using two-choice drug discrimination paradigms have shown that AMPH and FEN have overlapping DS effects (3, 7, 16). For instance, for pigeons trained to discriminate AMPH (2.0 mg/kg) from saline, FEN substituted in two pigeons at doses of 3.0 to 17 mg/kg and produced a maximum of 58% AMPH-appropriate responding at 10 mg/kg in a third pigeon. For the fourth pigeon, FEN failed to produce AMPH-appropriate responding up to a dose (10 mg/kg) that completely suppressed responding (7).

Once a successful discrimination was obtained among AMPH, FEN and saline using a three-choice drug discrimination procedure, phenmetrazine, which is similar to AMPH in terms of its central stimulant properties as well as its biochemical mechanism of action (4), was evaluated. Not surprisingly, in two-choice drug discrimination procedures, this drug has been shown to substitute for AMPH in several species (3, 7, 9). Likewise, phenmetrazine produced a dose-related increase in AMPH-appropriate responding in the present study with no responding on the FEN-appropriate key. Furthermore, the doses that substituted completely were in the same dose range under both conditions. To our knowledge this is the only study in which phenmetrazine has been evaluated in either a three- or two-choice FEN discrimination. Nonetheless, these results with phenmetrazine indicate that a three-choice drug discrimination procedure is comparable to two-choice drug discrimination procedures.

Quipazine and MK 212 (5-HT_2 and 5-HT_1 agonists, respectively) were both evaluated with the assumption that they would produce predominantly FEN-appropriate DS effects. Both quipazine and MK 212 have been shown to substitute for FEN in rats (34). Furthermore, several studies have demonstrated that the DS properties of quipazine and MK 212 are serotonergically mediated (5, 35, 36, 39). Another study (40) compared serotonin binding (5-HT_1 and 5-HT_2) in pigeon brain to results reported in rat brain (28,40). These binding results indicate that the pigeon has 5-HT_1 and 5-HT_2 binding sites and that these binding sites are comparable to those in the rat. In the present study quipazine produced FEN-appropriate responding in 2 of 3 pigeons, while a third pigeon responded predominantly on the AMPH-appropriate key at the highest dose (5.6 mg/kg). However, there is some evidence that quipazine may interact with central DA receptors (17,18). Furthermore, in a study by Schechter and Concannon (30), quipazine (1.0 mg/kg) substituted for the DA agonist, apomorphine, and the DS effects of quipazine were antagonized by the serotonin antagonist methysergide as well as the dopamine antag-

TABLE 2

RESPONSE RATE* FOLLOWING MDA, (+)-MDA, (−)-MDA AND MDMA

Drug	Dose (mg/kg; IM)	Pigeon 3215	4704	7216
MDA	0.3	2.87	1.78	2.39
	1.0	2.06	1.49	2.08
	1.7	2.26	1.95	—
	3.0	0.02	1.72	2.03
	5.6	—	0.00	0.81
	10.0	—	—	0.07
(+)-MDA	0.1	2.65	1.77	—
	0.3	2.53	1.68	—
	1.0	1.71	1.76	2.38
	1.7	1.79	1.78	2.32
	3.0	0.10	1.47	1.59
	5.6	—	0.00	0.49
(−)-MDA	0.1	—	1.46	—
	0.3	2.52	1.39	2.61
	1.0	1.88	1.34	1.74
	1.7	1.50	0.81	1.61
	3.0	0.45	0.27	0.83
	5.6	—	—	0.00
MDMA	0.1	—	1.90	—
	0.3	2.59	1.93	—
	1.0	2.40	1.98	2.37
	1.7	—	1.52	2.14
	3.0	1.91	1.58	1.74
	5.6	1.68	1.18	1.88
	10.0	—	1.03	0.00

*Response rate is expressed as responses/second.

onist haloperidol. In view of this evidence, it is not necessarily surprising that for animals trained to discriminate between an indirect DA agonist (AMPH) and an indirect serotonin agonist (FEN), some responding would occur on the AMPH-appropriate key when tested with quipazine. The other 5-HT agonist, MK 212, produced predominantly FEN-appropriate responding in two pigeons tested, confirming previous results in a drug vs. nondrug discrimination in rats (34). However, in a third pigeon, MK 212 produced predominantly AMPH-appropriate responding. While AMPH has not been evaluated in a two-choice MK 212 vs. saline discrimination, the DA agonist, apomorphine, was tested and it produced an average of 46% MK 212-appropriate responding (5). This suggests that MK 212, like quipazine, may have some DA actions.

In drug discrimination paradigms, some drugs are tested which are known to be pharmacologically unrelated to the training drug in order to confirm the pharmacological specificity of the discrimination. Evidence of such pharmacological specificity is the demonstration that these drugs produce saline-appropriate responding. Such specificity has been demonstrated previously in three-choice drug discrimination procedures (37,38). In pigeons trained to discriminate AMPH in a two-choice procedure (7), PCP failed to produce greater than 17% AMPH-appropriate responding with the exception of one pigeon that responded 43% on the AMPH-appropriate key at 1.0 mg/kg. Likewise, in the present study, PCP produced predominantly (greater than 50%) saline-appropriate responding at every dose tested in three pigeons, indicating that the DS effects of PCP were unlike either AMPH or FEN. Furthermore, in both studies, PCP was tested across a similar dose range (0.1–1.0 or 3.0 mg/kg) and the highest dose(s) produced similar rate decreases.

A potential advantage of three-choice drug discriminations is in the investigation of the DS properties of compounds with multiple neurochemical effects. For instance, MDA at doses between 1.0 and 5.6 mg/kg has been shown to substitute for both AMPH (6, 12, 24) and DOM (12) in two-choice drug discriminations indicating that this drug has both catecholaminergic and serotonergic effects. In the present study, MDA across a similar dose range also produced both AMPH-like and FEN-like DS effects again demonstrating that multiple neurochemical systems are involved in its DS effects. MDMA has also been shown to have multiple DS effects in two-choice drug discrimination procedures. For instance, in most studies, MDMA has been shown to substitute for AMPH (6, 11, 24), but Schechter (29) also demonstrated that MDMA substituted for FEN. Likewise, MDMA produced both AMPH- and FEN-appropriate responding in the present study. These results suggest that drugs which show evidence of multiple DS properties in two-choice drug discrimination procedures also show complex DS effects in a three-choice drug discrimination procedure. But unlike two-choice drug discrimination studies, evidence of multiple actions in the present study was obtained simultaneously. Consequently, the influence of potentially confounding variables such as different animals, different training histories, and different experimental procedures can be ruled out in the present study. Thus, it is possible to conclude that drug discrimination studies clearly support neurochemical evidence suggesting that MDA and MDMA have effects on both the catecholaminergic and serotonergic systems.

The utility of the present three-choice procedure is less clear in regards to the isomers of MDA. Results from two-choice drug discrimination studies indicate that (+)-MDA has DS properties similar to AMPH (11) but not to the hallucinogen DOM, which appears to be serotonergically mediated (13). These results were not completely confirmed in the present study. (+)-MDA did produce AMPH-appropriate responding in 2 of 3 pigeons but, in one of these pigeons, responding shifted from the AMPH-appropriate key at lower doses to the FEN-appropriate key at higher doses. In addition, predominantly FEN-appropriate responding occurred in a third pigeon. In contrast, (−)-MDA has been shown to substitute for DOM (13), mescaline and LSD (2) but not for AMPH (11) in two-choice drug discrimination procedures, but in the present study (−)-MDA produced responding on both drug keys in all three pigeons. However, given the small number of animals tested in the present study, the implications of these inconsistent findings cannot be determined in the absence of more extensive evaluations.

In summary, the results of the present study demonstrate that a mutually exclusive discrimination can be obtained between AMPH and FEN in a three-choice drug discrimination procedure. In general, drugs similar in terms of their behavioral effects and/or neurochemical mechanisms of action to either AMPH or FEN produced a DS profile similar to that obtained in two-choice drug discrimination procedures. The failure of PCP and lower doses of the test compounds to substitute for either AMPH or FEN provides evidence of the pharmacological specificity and sensitivity of the discrimination. Finally, the finding that MDA and MDMA which have effects on both catecholamine and serotonin receptor systems substitute for both AMPH and FEN in different pigeons as well as in the same pigeon strongly suggest that both actions contribute to their DS simultaneously, an interpretation that is not possible when two-choice drug discrimination procedures are used. However, the mixed results with the isomers of MDA indicate the need for additional studies designed to elucidate the relative contribution of different neurochemical systems in the DS effects of these drugs.

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