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Effects of Opiates on the Discriminative Stimulus Properties of Dopamine Agonists

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HERNANDEZ, L. L., A. M. HOLOHEAN, AND J. B. APPEL. *Effects of opiates on the discriminative stimulus properties of dopamine agonists.* PHARMAC. BIOCHEM. BEHAV. 9(4) 459–463, 1978.—Two groups of rats were trained to discriminate the dopamine agonists amphetamine (0.75 mg/kg) or apomorphine (0.38 mg/kg) from saline in a two-lever operant task. Various doses of morphine and pentazocine were tested for generalization to and interference with the discriminative stimulus complexes produced by the dopamine agonists. Low doses of morphine appeared to produce a stimulus complex which is similar to that produced by apomorphine, but which differs from that produced by amphetamine. Pentazocine showed no evidence of generalization to either the apomorphine or the amphetamine cue. Neither opiate interfered with the discriminative stimuli produced by the dopamine agonists, although decreases in the number of animals responding occurred.

Dopamine agonists d-Amphetamine Apomorphine Morphine Pentazocine
Discriminative stimulus properties of drugs Mechanism of action Rats

IN RECENT years central dopaminergic mechanisms have been implicated in a variety of opiate effects [13, 14, 15, 17, 18]. Available data indicate that blockade of central dopamine receptors with haloperidol reduces morphine self-administration in rats [10,22] and is somewhat effective in suppressing opiate craving in human heroin addicts [12]. However, haloperidol neither mimics [8] nor suppresses [1, 5, 9] the discriminative stimulus complex (DS) produced by opiates in rats. Since the DS in animals may be related to the subjective effects of opiates and other drugs of abuse in man [2, 16, 19] its pharmacological characterization with respect to central dopaminergic mechanisms is of considerable applied as well as theoretical importance.

Two other drugs capable of producing a DS in rats are amphetamine [20] and apomorphine [6]. Although not necessarily similar to one another, each of these DS's is related to central dopaminergic stimulation since each can be antagonized with haloperidol [6, 11, 21]. Neither amphetamine nor apomorphine produces drug-like responding in rats trained to discriminate morphine from saline [9,23] or fentanyl from saline [2], although fentanyl does produce drug-like responding in rats trained to discriminate apomorphine from saline—an effect that can be blocked by haloperidol [5]. Thus, although blockade of central dopamine receptors does appear to alter the "reinforcement value" of opiates in both rats and humans, the mechanism of dopaminergic involvement in the opiate DS has not yet been clearly elucidated.

The present study compares the effects of morphine and pentazocine, a mixed agonist-antagonist analgesic whose DS properties have been shown to be partially mediated by dopaminergic stimulation [1], in rats trained to discriminate either amphetamine or apomorphine from saline. Since the DS properties of amphetamine are thought to be produced by release of dopamine (DA) from presynaptic terminals [11] whereas those of apomorphine are presumed to be the result of direct postsynaptic DA receptor stimulation [3,6], it was hoped that differential effects on the two discriminations would help characterize the nature of the dopaminergic mechanisms of the opiates studied. The results, although preliminary, suggest that the stimulus complex produced by morphine includes a component that is similar to the DS produced by apomorphine; pentazocine did not generalize to either the amphetamine or the apomorphine DS. (Completion of this study as originally planned was prohibited when the building housing this laboratory partially burned. Portions of the results are currently being replicated and extended.)

METHOD

Animals

Twelve 250–300 g, experimentally-naive male albino rats of the Sprague-Dawley strain (obtained from Charles River Breeding Laboratories, Wilmington, Massachusettes) were used. They were maintained at 80% of their free-feeding weights by restricting water intake, and were housed individually in a room maintained at constant temperature (23°C) and humidity (40%), on a 12-hr light-dark cycle (7:00 a.m.–7:00 p.m.). Lab chow was always available in home cages.

Apparatus

Four, two-lever operant chambers (Lehigh Valley Electronics Model 143–25), housed in ventilated and sound-attenuating shells (LVE Model 132–04) were used. Each chamber was equipped with 2 levers located on one wall,

separated by a liquid dipper which delivered 0.10 ml of tap water per reinforcement. Electromechanical programming and recording equipment were located in an adjoining room.

Procedure

The animals were randomly divided into 2 groups of 6 rats each. One group (AMP) was trained to respond differentially on 1 of the 2 levers on a fixed-ratio reinforcement schedule (FR 32) depending on whether d-amphetamine (0.75 mg/kg) or saline had been injected intraperitoneally (IP) 30 min prior to the beginning of the session. The other group (APO) was similarly trained to respond differentially depending on whether apomorphine (0.38 mg/kg) or saline had been injected 30 min prior to the beginning of the session. The position (right or left) of the lever designated as correct following training drug administration was counterbalanced within groups.

On the first 2 days of training all animals received 1.0 ml/kg of saline IP; the appropriate drug lever was removed from the chambers, and pressing the saline lever was shaped, initially on an FR 1 (continuous reinforcement) schedule. The FR requirement was gradually raised until all animals performed on FR 32. On the next 2 days, the animals received either 1.25 mg/kg of amphetamine or 0.50 mg/kg of apomorphine; the saline levers were removed and the animals were trained to press the drug-appropriate lever on FR 32. This double alternation of training drug and saline injections was repeated until all animals were responding consistently and at a high rate on both levers. Both levers were then placed in the chambers and pressing the correct lever was reinforced, depending on prior drug or saline administration. Percent correct responding was taken as the percentage of the total number of responses made prior to completion of the first reinforced sequence on the correct lever. The double alternation schedule of drug and saline injections continued until baseline response rates had stabilized on each lever and mean percent correct for each group for each drug condition attained a criterion of 85%. The training drug doses were gradually lowered to 0.75 mg/kg for the AMP group and 0.38 mg/kg for the APO group.

When each group again attained the 85% correct criterion for each drug condition, a weekly schedule was instituted under which all animals were given saline on Mondays and Thursdays and the appropriate training drug on Wednesdays, followed by the usual 30 min training session. Tuesdays or Fridays were designated as test days: on these days, animals received either a test drug in combination with saline (transfer tests) or a test drug in combination with the training drug (antagonism tests). The test drug combinations are summarized in Tables 1 and 2. When test drugs were administered 30 min prior to testing, they were given with the appropriate training substance as a single injection. When test drugs were given at other times, the appropriate training substance was given as a second injection 30 min prior to testing. All drug administrations were given IP in a volume of 1.0 ml/kg.

All training sessions lasted 30 min; test sessions were conducted under extinction conditions, and continued until the animal had completed 32 responses on a single lever. Animals were then removed from the test chamber, and returned to home cages where they were allowed 5 min free access to water. Animals received no water other than that obtained as reinforcement on training days; on weekends animals were allowed free access to water from 1 p.m. Friday until 5 p.m. Saturday.

Data Analysis

Percent drug lever responding for each animal for each training and testing session was taken as a percentage of the total number of responses made on both levers prior to completion of the first FR 32 on a single lever. Only animals completing at least 8 lever-presses during the 30 min experimental sessions were considered as having responded; data from the 2 groups (AMP and APO) were treated separately. One-tailed *t*-tests were performed to determine if the group mean difference between drug-lever responding following experimental treatments and that of the immediately preceeding saline session differed significantly from zero. One-tailed *t*-tests were also used to assess the reliability of mean differences in performance between that on the test day and that on the training-drug day of the same week. If test day performance differed from both saline and drug controls, two-tailed *t*-tests were performed to determine if mean test day performance differed significantly from chance responding. Differences with probable reliability greater than 95% were considered significant; differences with probable reliability greater than 90% were considered marginally significant, and as indicating the performance of tests versus chance. Only data from those animals that responded on all 3 relevant days (saline and drug controls and the test day) were included in the analysis.

The drugs used in this study were: apomorphine hydrochloride (Merck), d-amphetamine sulfate (Sigma), morphine sulfate (Lilly), pentazocine ampules (Talwin; Sterling-Winthrop), haloperidol ampules (Haldol; McNeil), and naltrexone hydrochloride (Endo). All drugs were dissolved in 0.9% saline such that concentration (in mg/ml) was equal to the dose administered (in mg/kg). The doses of all drugs refer to their salts.

RESULTS

Baseline response parameters for the AMP and APO groups are reported in Tables 1 and 2, and reflect the overall mean percent drug lever responding of all animals that responded on each saline and drug control day (including animals that failed to respond on the test day). Both discriminations were learned, with the AMP group requiring a total of 36 training sessions to criterion, while the APO group required a total of 77 training sessions prior to the beginning of testing. As can be seen in Tables 1 and 2 both groups showed consistently high accuracy of discrimination across control sessions, although discrimination in the APO group was somewhat lower and more variable than that in the AMP group. While this may be partially due to the fewer number of control days for the APO group, these data suggest that, at the doses used, apomorphine was less discriminable from saline than was amphetamine.

The effects of the experimental treatments on mean percent drug lever responding are also summarized in Tables 1 and 2. Means, standard errors and probabilities are based only on those animals that responded on the test day; the numbers of animals completing more than 50% of their responses on the drug lever are also presented for comparison. In the cross-generalization tests both apomorphine (Table 1) and amphetamine (Table 2) produced responding that differed from both saline and training drugs. The DA antagonist

TABLE 1

EFFECTS OF PSYCHOACTIVE DRUGS ON RATS TRAINED TO DISCRIMINATE AMPHETAMINE (0.75 MG/KG) FROM SALINE

Drug	Time*	Dose (mg/kg)	Test	%†	SEM	p vs. SAL	p vs. Drug	p vs. Chance	Animals Responding	Animals Choosing AMP Lever‡
SAL	30			2.9	0.7§					
AMP	30	0.75		94.9	1.5§					
APO	30	0.25	T¶	29.0	15.9	(0.10)	0.005	n.s.	6/6	1/6
	30	0.50	T	50.8	20.9	0.05	0.05	n.s.	6/6	3/6
HAL	60	0.05	A#	89.4	10.0	0.001	n.s.		6/6	5/6
MORPH	20	5.0	T	3.0	1.7	n.s.	0.001		3/5	0/3
	30	5.0	T	14.5	13.1	n.s.	0.05		5/5	1/5
	40	5.0	T	50.7	18.6	0.05	0.05	n.s.	5/5	3/5
	60	5.0	T	39.9	19.8	(0.10)	0.05	n.s.	4/5	1/4
	90	5.0	T	4.9	3.2	n.s.	0.001		4/5	0/4
	30	7.5	T	9.0	9.0				2/5	0/2
MORPH	20	5.0	A	82.7	17.3	0.01	n.s.		4/5	3/4
	40	5.0	A	98.0	2.0	0.005	n.s.		3/5	3/3
	60	5.0	A	62.8	37.2				2/5	1/2
	60	2.5	A	72.7	27.4				2/5	1/2
PENT	20	10.0	T	12.5	7.6	n.s.	0.001		5/5	0/5
	30	10.0	T	0.6	0.6	n.s.	0.005		5/5	0/5
	40	10.0	T	30.5	16.7	n.s.	0.01		5/5	2/5
PENT	20	10.0	A	100.0	—	0.001	n.s.		4/5	4/4
	40	10.0	A	70.4	29.6	(0.10)	n.s.		3/5	2/3
NAL	60	1.0	A	90.0	5.0	0.001	n.s.		5/5	5/5

*Minutes before testing.
†Mean percent responding on AMP-appropriate lever.
‡Completing >50% of responses on AMP-appropriate lever.
§Overall mean of control days ± standard error of 19 means.
¶Transfer.
#Antagonism.

haloperidol (0.05 mg/kg) completely blocked the apomorphine DS (Table 2) and, at this dose, had little effect on the amphetamine DS (Table 1). This result is in agreement with those of Schechter and Cook [21] who found that higher doses of haloperidol were required to block the amphetamine DS than were required to block the apomorphine DS.

Tests for transfer of the stimulus complexes produced by morphine and pentazocine showed that, in the AMP group (Table 1), 5.0 mg/kg of morphine, 40 to 60 min prior to testing, elicited responding that differed from both saline and amphetamine controls; 5.0 mg/kg of morphine 20 to 30 min and 90 min prior to testing produced saline-like responding. When 7.5 mg/kg of morphine was administered 30 min prior to testing only 2 rats responded, although both responded primarily on the saline lever. Pentazocine (10.0 mg/kg), 20 to 40 min prior to testing produced only saline-like responding (Table 1). In the APO group morphine (5.0 mg/kg, 30 min prior to testing) produced drug-like responding whereas 5.0 mg/kg of pentazocine, 30 min prior to testing, produced saline-like responding (Table 2).

When opiates were given in combination with the usual training drug (antagonism tests) little evidence for antagonism of the DSs produced by the DA agonists was obtained, although a decrease in the number of rats responding occurred. When given in combination with amphetamine 5.0 mg/kg of morphine 20 to 40 min prior to testing had no effect on the AMP DS; a decreasing number of animals responded as the time between morphine administration and testing increased (Table 1). When 2.5 or 5.0 mg/kg of morphine was given 60 min prior to testing in combination with amphetamine only 2 rats responded, although in each case one of the two animals responded primarily on the saline lever. Similarly, 10.0 mg/kg of pentazocine administered 20 to 40 min prior to testing failed to reliably affect the amphetamine DS, although again the number of animals responding decreased as the time between pentazocine injection and testing increased. Naltrexone (1.0 mg/kg, 60 min prior to testing) in combination with the usual amphetamine injection also failed to alter the amphetamine DS.

When 5.0 mg/kg of morphine was given in combination with apomorphine (Table 2) only 1 rat in the APO group responded; in this animal all responses occurred on the drug

TABLE 2

EFFECTS OF PSYCHOACTIVE DRUGS ON RATS TRAINED TO DISCRIMINATE APOMORPHINE (0.38 MG/KG) FROM SALINE

Drug	Time*	Dose (mg/kg)	Test	%†	SEM	*p* vs. Sal	*p* vs. Drug	*p* vs. Chance	Animals Responding	Animals Choosing APO Lever‡
SAL	30			4.1	4.2§					
APO	30	0.38		90.8	10.8§					
AMP	30	0.75	T¶	55.3	15.3	0.01	0.025	n.s.	6/6	3/6
	30	1.25	T	50.1	17.5	(0.10)	0.05	n.s.	4/6	3/4
HAL	60	0.05	A#	17.1	15.5	n.s.	0.025		6/6	1/6
MORPH	30	5.0	T	84.7	6.1	0.001	n.s.		4/4	4/4
	30	5.0	A	100.0					1/4	1/1
PENT	30	5.0	T	20.8	19.3	n.s.	0.05		3/4	1/3
	30	5.0	A	99.0	1.0	0.001	n.s.		3/3	3/3

*Minutes before testing.
†Mean percent responding on APO-appropriate lever.
‡Completing >50% of responses on APO-appropriate lever.
§Overall mean of control days ± standard error of 7 means.
¶Transfer.
#Antagonism.

lever. Similarly 5.0 mg/kg of pentazocine, 30 min prior to testing failed to reliably alter the apomorphine DS in those animals that were tested.

DISCUSSION

The results of the cross-generalization tests of the present experiment suggest that, at the doses used, the stimulus complexes produced by amphetamine and apomorphine are not similar (i.e., they did not generalize to each other) but share a common (presumably dopaminergic) basis which served to distinguish each drug from no drug (i.e., they did not generalize to saline); it has been shown [21] that higher doses of these drugs may produce more complete cross-generalization. The results for the AMP group also agree closely with data reported by Ho and Huang [11] who, using similar doses, found a dose-related increase in amphetamine-lever responding following increasing doses of apomorphine, up to but not surpassing chance levels. The relationship between the amphetamine and the apomorphine DS may be reciprocal, in that amphetamine produced apomorphine-lever responding greater than that after saline but less than that after apomorphine, although no dose relationship is apparent in the present results. Further investigation would be needed to clarify the relationship between the DSs produced by these two DA agonists.

Although they must be considered preliminary, the results suggest that the stimulus complex produced by 5.0 mg/kg of morphine is similar to that of apomorphine (0.38 mg/kg) and different from that of amphetamine (0.75 mg/kg). While morphine administered to the AMP group 40 to 60 min prior to testing did produce responding that differed from both amphetamine and saline controls, it is evident that generalization of the morphine stimulus complex to the amphetamine DS is, at best, weak and restricted to a short period of time beginning about 30 min after injection. Increasing the dose of morphine to 7.5 mg/kg resulted only in decreasing the number of animals that responded, but did not increase generalization to the amphetamine DS. It is interesting to note, however, that Shannon and Holtzman [23] have reported that 0.1 to 3.0 mg/kg of amphetamine administered to rats trained to discriminate 3.0 mg/kg of morphine from saline produced a dose-related increase in morphine-lever responding up to but not surpassing chance levels.

5.0 mg/kg of morphine administered to the APO group 30 min prior to testing did produce complete transfer to the drug-appropriate lever. This result is in agreement with those of Colpaert *et al.* [5] using fentanyl, and supports their conclusion [4,5] that the stimulus complex produced by opiate agonists includes a component related to postsynaptic DA receptor stimulation.

While the results obtained with pentazocine must be considered inconclusive because of the limited number of tests given and the different dosages administered to the two groups, it is evident that, at the doses used, the stimulus complex produced by pentazocine is not similar to the DSs produced by the DA agonists. The findings of Appel *et al.* [1] that the DS produced by pentazocine could be only partially blocked by either naltrexone or haloperidol, but could be completely blocked by a combination of naltrexone and haloperidol, indicates that pentazocine's DS is related in part to dopaminergic stimulation. The present results show no evidence that the stimulus complex produced by pentazocine generalizes to the DS produced by either amphetamine or apomorphine, although higher doses of pentazocine may show such generalization.

The results of the antagonism tests show little evidence of interference with the amphetamine or apomorphine DSs by the opiates. This is in contrast to results involving other behavioral tests which show antagonism between the effects of opiates and those of DA agonists [7]. The combination of the opiates with the DA agonists did interfere with respond-

ing, particularly in the case of morphine. The time course of this depression of responding in the AMP group remains unexplained but may reflect motor, stereotyptogenic or toxic interactions of the drugs. The nature of these interactions awaits further investigation.

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