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Discriminative Properties of *l*-Cathinone Compared to *dl*- and *d*-Cathinone[1]

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SCHECHTER, M. D. *Discriminative properties of* l-*cathinone compared to* dl- *and* d-*cathinone*. PHARMACOL BIOCHEM BEHAV **24**(5) 1161–1165, 1986.—Rats were trained to discriminate between the stimulus properties of 0.6 mg/kg *l*-cathinone and its vehicle in a two-lever, food-motivated operant task. Once trained, rats showed a dose-dependent decrease in discrimination performance with lower *l*-cathinone doses and analysis of the dose-response relationship indicated an ED50 of 0.19 mg/kg. Administration of either *dl*- or *d*-cathinone produced a pattern of discriminative responding similar to *l*-cathinone with ED50s of 0.29 and 0.63 mg/kg, respectively. Thus, the potency of the racemeric cathinone lies approximately midway between that of the two isomers. Time-course data indicate that *l*-cathinone has a peak effect at 15–30 min post-administration with a duration of 180 min. Pretreatment with the serotonic receptor blocker pirenperone did not affect *l*-cathinone discrimination, whereas pretreatment with 0.2 mg/kg haloperidol, a dopamine receptor blocking agent, attenuated the *l*-cathinone discrimination. These data suggest that the stimulus properties of *l*-cathinone are possibly mediated by brain dopaminergic systems.

Drug discrimination Cathinone Isomers Dopamine Antagonism Stimulus properties of drugs Haloperidol Pirenperone

CATHINONE (alpha-aminopropiophenone) has been established as the active psychostimulant component of the khat plant (*Catha edulis* Forsk.) [21] and *l*-cathinone has been shown to be structurally similar to *d*-amphetamine [2]. In this laboratory [5, 17, 18] and elsewhere [9], cathinone has been employed as a drug capable of controlling discriminative behavior in rats. The cathinone-induced discriminative cue generalizes to amphetamine [18] and vice versa [14].

Consistent with the results of other studies on the isomers of cathinone [6,14], *l*-cathinone has been shown to be more potent than its enantiomer in this behavioral paradigm [5]. In light of the fact that the *l*-isomer of cathinone is the active constituent of the khat plant [11], the purpose of the present study was to train rats to discriminate the stimulus effects of *l*-cathinone and to compare dose-response and time-course effects of administration of its racemate and enantiomer in these rats. In addition, clarification of the neurochemical mechanisms of the discriminative effects of *l*-cathinone was investigated by using specific dopaminergic and serotonergic blockers.

METHOD

Subjects

The subjects were 10 male ARS/Sprague-Dawley rats weighing 670–800 g at the beginning of experimentation. They were individually housed in galvanized cages with free access to tap water except during experimental sessions. Their weights were adjusted, by daily rationing of commercial rat chow, to approximately 80 to 85% of their expected free-feeding weights as determined by daily weighing of 2 control free-feeding rats purchased from the supplier (Zivic-Miller, Allison Park, PA) at the same time as the experimental subjects. Room lights were on from 0600 to 1800 in a room with a constant temperature of 20–22°C.

Apparatus

The experimental space consisted of 8 identical standard rodent operant test cages (Lafayette Instruments Corp., Lafayette, IN) each equipped with 2 levers located 7 cm apart and 7 cm above the gridded floor. A food pellet receptacle was mounted 2 cm above the floor at an equal distance between the levers and food delivered into this cup consisted of a single 45 mg food pellet (Bioserv Inc., Frenchtown, NJ). The test cage was housed in a sound-attenuating cubicle equipped with an exhaust fan and a 9 W house-light. Solid-state programming equipment (Med Associates, E. Fairfield, VT) was used to control and record the sessions and was located in an adjacent room.

Discrimination Training

Training was based upon procedures described elsewhere [18]. There were two training phases. In the first phase,

[1]Preliminary aspects of this study were presented at the Canadian College of Neuropsychopharmacology meeting in London, Ontario in June, 1985.

food-deprived subjects were trained to lever press on both levers for food reinforcement (45 mg Noyes pellets) on a fixed ratio 10 (FR10) schedule. The saline-appropriate lever was activated first for all subjects. The rats were trained, by successive approximations, to press this lever on a FR1 schedule. The fixed ratio requirement was progressively increased, in daily 15 min sessions, over 10 days until an FR10 schedule was achieved. Throughout lever press training, rats received daily intraperitoneal (IP) injections of saline (0.9% sodium chloride) 15 min prior to being placed into the two-lever operant box. Immediately following attainment of the FR10 schedule after saline adminstration, the opposite lever was activated and rats were trained on a FR1 schedule after the IP administration of an equal volume of saline (1 ml/kg body weight) containing 0.6 mg/kg *l*-cathinone. Daily sessions of 15 min were continued over 8 days with cathinone administration until an FR10 schedule was attained. In order to minimize effects due to any possible position preference, the 10 rats were divided into 2 groups. For one group, responding on the left lever was reinforced by delivery of food pellets in every session following drug injection, whereas the other group was reinforced for responding on the right lever following drug injections. Responses on the opposite lever were reinforced with food pellets after saline injections and the running order was randomized amongst the 8 chambers.

Phase II discrimination training then began. Subjects were trained 5 days per week with alternation of reinforcement in a pseudo-random sequence. Thus, in each 2 week period, there were 5 days with drug lever (D) correct and 5 days with saline lever (S) correct. The pattern was D,S,S,D,D; S,D,D,S,S. Due to the varied sensitivity of individual rats to drug training in the past [15], it was decided to modify previously employed [18] criteria for training to insure that an animal was, in fact, trained to the cathinone-induced discriminative stimulus. This modification in protocol required that an animal perform a series of *two* sets of ten consecutive trials with a 9 of 10 accuracy in a given session before it was allowed to be used for data collection. During these experiments, one rat died of unrelated cause(s) and the results reflect an n=9.

Dose-Response Relationships to l-Cathinone

Once these animals attained the second required training set achieving 9 of 10 consecutive correct sessions, they were tested for their sensitivity to lower doses of *l*-cathinone. Testing and training sessions of 15 min duration with alternating administrations of 0.6 mg/kg cathinone and saline were continued on Mondays, Wednesdays, and Fridays. This procedure endeavored to insure and maintain behavioral discrimination of the training drug conditions, and it was lever selection during these maintenance trials that was employed to generate those values at 0.6 mg/kg *l*-cathinone and saline. It was intended that if a rat was observed to make more than 2 incorrect lever selections before making 10 correct selections in any of 10 consecutive maintenance sessions, the data on that rat's performance would be deleted from the results. This, however, did not occur. On Tuesdays and Thursdays, the rats were injected IP with different doses of cathinone than used for initial training, i.e., 0.3 and 0.15 mg/kg and, 15 min later, they were placed into the experimental chamber and were allowed to lever press, in extinction, until 10 responses were made on either lever. To preclude training at a cathinone dose different than the 0.6 mg/kg dose employed to train the animals, the rats were immediately removed from the experimental chamber upon making 10 responses on either lever. Each of the two lower doses of cathinone were tested in each animal on two occasions with each test preceded both by a 0.6 mg/kg cathinone and a saline maintenance session. The lever first pressed 10 times was designated as the "selected" lever (below).

Time-Course of Drug Action

Following the dose-response experiments, testing days were used to test the time-course of action of the training dose (0.6 mg/kg) of *l*-cathinone. Thus, the rats were administered 0.6 mg/kg *l*-cathinone IP and they were tested at various times after drug administration. All post-injection times were randomized and each time was preceded by both a maintenance saline and a cathinone session at 15 min post-injection. Post-injection times of 30, 45, 60, 90, 120 and 180 min were employed and at the end of each of these times, the rats were removed from their home cage, placed in the test chamber and allowed to choose one of the two levers without reinforcement. Once either lever was pressed 10 times, the rat was immediately removed from the test chamber to preclude further training.

Generalization to dl- *and* d-*Cathinone*

Subsequently, each rat was injected on two test sessions with one of several doses of *dl*-cathinone (0.15–0.6 mg/kg) or *d*-cathinone (0.6–1.2 mg/kg) to test for generalization or transfer of the *l*-cathinone-produced discriminative stimulus to its racemer and enantiomer. As before, all test sessions were preceeded by interspersed maintenance sessions and conducted in a random order. To preclude training with a drug different than *l*-cathinone, rats were removed immediately upon making 10 responses upon either lever.

Antagonism With Haloperidol and Pirenperone

Test days were subsequently used to test the effects of pretreatment with haloperidol and pirenperone prior to injection of saline and the 0.6 mg/kg training dose of *l*-cathinone. Haloperidol, at a dose (0.2 mg/kg) previously observed to antagonize *d*-amphetamine discrimination in a similar paradigm [16], was administered 15 min prior to *l*-cathinone and the rats were tested 15 min after the second injection. Likewise, pretreatment with the serotonin antagonist pirenperone at a dose (0.16 mg/kg) that has been reported to antagonize LSD discrimination [3], was tested 15 min prior to saline or *l*-cathinone administration. In all cases, the rat was allowed to press either of the two levers and it was immediately removed upon making 10 responses on either lever.

Drugs

The hydrochloride salts of *dl*-, *d*- and *l*-cathinone were supplied by Dr. Richard Hawks of the National Institute of Drug Abuse. These drugs were dissolved in saline with doses calculated as the salt and were administered IP in an equal volume of 1 ml/kg 15 min prior to testing (except in time-course experiments). Haloperidol (McNeil) was diluted from ampules to 0.2 mg/ml and pirenperone (Janssen) was dissolved in saline.

Measurements

The lever pressed 10 times first was designated as the

TABLE 1

DOSE-RESPONSE RELATIONSHIPS TO *l*-, *dl*-, AND *d*-CATHINONE IN RATS TRAINED TO DISCRIMINATE *l*-CATHINONE

Dose (mg/kg)	*l*-cathinone		*dl*-cathinone		*d*-cathinone	
	Quantal	Quantitative (SD)	Quantal	Quantitative (SD)	Quantal	Quantitative (SD)
1.2	ND		ND		94.4	80.7 (4.2)
0.9	ND		ND		83.3	76.7 (3.0)
0.6	100.0	92.9 (3.8)	83.3	79.5 (21.6)	44.4	53.8 (26.1)
0.3	77.8	68.6 (0.7)	38.9	41.2 (5.0)	ND	
0.15	33.3	40.5 (5.9)	27.8	33.7 (10.4)	ND	
ED50: (95% confidence limits)	0.19 (0.12–0.30)		0.29(0.18–0.47)		0.63 (0.47–0.85)	

ND: Not determined.

"selected" lever. The percentage of rats selecting the lever appropriate for the training drug was the quantal measurement of discrimination. In addition, the number of responses on the cathinone-correct lever divided by total responses made prior to 10 responses (including the ten on the cathinone-correct lever) times 100 constitutes the quantitative measurement. The advantages in using both measurements have been discussed by Stolerman and D'Mello [20]. The quantal data for the dose-response experiments were analyzed by the method of Litchfield and Wilcoxon [12] which employs probit vs. log-dose effects and generates ED50s and tests for parallelism. The quantitative measurements were compared by a Student's *t*-test of means with $p<0.05$ chosen as the level of significance.

RESULTS

Acquisition of Discrimination

The 10 rats required a mean (±S.D.) of 15.7 (8.0) sessions to the first set of criterion performance, i.e., to the first of 9 of 10 consecutive sessions in which the correct lever was selected. The second set of criterion performance was achieved at a mean of 25.7 (8.0) sessions. Thus, all rats learned to discriminate 0.6 mg/kg *l*-cathinone by the 50th session (25 training trials with each state).

Dose-Response to l-*Cathinone and Transfer to* dl- *and* d-*Cathinone*

Once training criterion was attained, decreasing doses of *l*-cathinone produced decreased discrimination performance both in terms of the quantal and quantitative measurements (Table 1, left column). Analysis of this dose-response relationship [12] indicated an ED50 (with 95% confidence limits) of 0.19 (0.12–0.30) mg/kg. Administration of similar doses of *dl*-cathinone likewise produced a dose-response relationship, with an ED50 of 0.29 (0.18–0.47) mg/kg, that was parallel to that observed with *l*-cathinone, i.e., the calculated $t=0.18<$critical $t=4.3$ [12]. The quantitative measurement after 0.3 mg/kg *l*-cathinone (68.6) was found to be significantly ($p<0.009$) higher than that observed after 0.3 mg/kg *dl*-cathinone (41.2).

Testing of 0.6–1.2 mg/kg *d*-cathinone indicated that rats trained to discriminate 0.6 mg/kg *l*-cathinone perceive the *d*-isomer in a similar fashion with decreasing doses producing decreased discriminative performance. Analysis of the dose-response relationship for *d*-cathinone [12] yielded an ED50=0.63 (0.47–0.85) mg/kg and the dose-response relationship was parallel to that of *l*-cathinone with the calculated $t=0.94<$critical $t=4.3$.

TABLE 2

TIME-COURSE OF ACTION OF *l*-CATHINONE

Time after 0.6 mg/kg *l*-cathinone IP (min)	Quantal	Quantitative (SD)
15	100.0	92.4 (3.3)
30	94.4	91.6 (7.4)
45	78.8	73.0 (1.2)
60	83.3	74.4 (4.2)
90	38.9	46.0 (7.4)
120	16.7	29.8 (25.5)
180	5.6	14.7 (2.9)
Saline 15	4.9	17.7 (4.2)

Time-Course of l-*Cathinone*

The results of testing *l*-cathinone at different post-administration times (Table 2) indicates that the discriminative performance generally decreases over time. Testing of 0.6 mg/kg *d*-cathinone at 180 min post-injection produced discrimination at levels similar to that observed after saline administration.

Antagonism With Haloperidol and Pirenpirone

Maintenance testing days with 0.6 mg/kg *l*-cathinone produced 96.7% of responses upon the cathinone-appropriate lever, whereas saline administration resulted in 7.9% responses on this lever (or 92.1% responses on the saline-correct lever). Pretreatment with 0.2 mg/kg haloperidol had no effect upon saline administration but decreased cathinone discrimination to 68.8% of total trials with a quantitative measurement significantly lower than that observed after cathinone discrimination. In contrast, pretreatment with 0.16 mg/kg pirenperone had no effect on either cathinone or saline discrimination (Table 3).

TABLE 3

DISCRIMINATION OF *l*-CATHINONE AND SALINE AFTER PRETREATMENT WITH HALOPERIDOL AND PIRENPERONE

Pretreatment	Dose (mg/kg)	Treatment	Dose (mg/kg)	Quantal	Quantitative (SD)
		l-cathinone	0.6	96.7	92.1 (5.0)
		saline	—	7.9	22.3 (5.7)
Haloperidol	0.2	*l*-cathinone	0.6	68.8	63.3 (4.9)*
		saline	—	5.6	26.5 (5.7)
Pirenperone	0.16	*l*-cathinone	0.6	100.0	94.8 (2.8)
		saline	—	5.6	22.0 (3.2)

*Significant difference ($p<0.00001$) from quantitative measurement after 0.6 mg/kg *l*-cathinone tested alone (*t*-test of means).

DISCUSSION

Analysis of freeze-dried leaves of khat plants by the United Nations Narcotic Laboratory in the early 1970s indicated that *l*-cathinone [(−)-S-α-aminopropiophenone] was the principal active constituent [2, 8, 21]. The present report indicates that *l*-cathinone, like its racemic mixture [5, 17, 18], can function as a drug to control discriminative behavior in the rat. This discrimination was observed to be dose-dependent over a range (0.15–0.6 mg/kg) of *l*-cathinone doses and when rats trained to discriminate *l*-cathinone are administered either *dl*- or *d*-cathinone they perceive these latter substances as *l*-cathinone.

Comparisons of the ED50s of the isomers and racemate of cathinone in this series of discrimination studies indicated that *l*-cathinone is approximately 1.5 times more potent than *dl*-cathinone and 3.0 more potent than the *d*-isomer. In addition, racemic cathinone produces discriminative performance about midway between the two isomers; a result that might have been predicted simply because the *dl*-racemate contains both *d*- and *l*-isomeric components. In studying the effect of these drugs on the locomotor activity of mice, Glennon and Showalter [6] likewise reported that administration of racemic cathinone displays the effects of both components and that *l*-cathinone was more active than its *d*-enantiomer and its *dl*-racemate. In addition, self-administration experiments in monkeys indicated that *l*-cathinone was more potent than *dl*-cathinone [19].

Time-course experiments indicated that the discriminative effects of *l*-cathinone peak between 15 and 30 min post-injection, remain at approximately 80% through 60 minutes, fall to random levels at 90 min and reach saline-appropriate response level by 180 min (Table 2). This time-course of action is similar to that seen in locomotor activity studies in mice where the peak effects are seen within 30 min [6,24] and in circling experiments in rats where peak effects occur within 60 min [24].

Numerous behavioral and neurochemical studies have indicated the similarity of effects of *l*-cathinone and *d*-amphetamine [6, 9, 10, 14, 18, 22, 25] and both drugs have recently been reported to decrease DOPAC levels and inhibit dopaminergic neuron firing rates in rat brain regions [13]. Thus, it appears that, like *d*-amphetamine, *l*-cathinone's actions are mediated by brain dopaminergic mechanisms. Further evidence for this finding is found in various studies in which known dopamine receptor antagonists attenuate the effects of *l*-cathinone. Thus, haloperidol reverses *l*-cathinone's action upon dopamine neuronal firing [13]; haloperidol, spiroperidol and pimozide block its locomotor enhancement effects [22] and haloperidol attenuates its production of stereotypy in rats [25]. In the present study, pretreatment with 0.2 mg/kg haloperidol was observed to significantly decrease the rats' ability to discriminate *l*-cathinone without a similar effect upon saline discrimination. Nevertheless, the rats selected the cathinone lever in 68.8% of trials after pretreatment with 0.2 mg/kg haloperidol. A higher dose of haloperidol was precluded by the onset of behavioral disruption. These findings are in contrast to those of Rosecrans *et al.* [14] and Huang and Wilson [9] who reported that haloperidol pretreatment, at doses of 0.1 mg/kg and 0.15 mg/kg, respectively, did not significantly decrease the discrimination of *dl*-cathinone. These discrepancies may be due to the dose level of haloperidol used, i.e., a higher (0.2 mg/kg) dose in the present experiment, or to the drug used to train rats to make differential responses, i.e., *l*- vs. *dl*-cathinone.

In addition to the many studies relating *l*-cathinone effects to brain dopaminergic neurons, there are two recent reports that indicate that cathinone may interact with serotonergic sites in the brain. One study [4], using the isolated rat fundus preparation, indicated that *l*-cathinone possesses twice the serotonin receptor affinity of *dl*-cathinone and four times the affinity of *dl*-amphetamine. The second report [1] demonstrated that cathinone decreases the differentiation of visual stimuli in cats and that this effect can be altered by serotonergic blockade with methysergide. The present results indicate that pirenperone, a newly synthesized and specific serotonin receptor blocker [7], did not affect the discrimination of *l*-cathinone at the dose used for pretreatment. This confirms one other recent study [9] in which the less specific serotonergic receptor blocker methysergide failed to alter the stimulus properties of *dl*-cathinone and similar observations that serotonin blockers [23] and synthesis inhibitors [16] do not alter the discrimination of *d*-amphetamine.

In conclusion, the results of the present study indicate that *l*-cathinone can function as a discriminative stimulus in rats in a dose-dependent manner with peak effects between 15 and 30 min and that the potency of the *l*-isomer is greater than either the *d*-isomer or the *dl*-racemate. In addition, these data suggest that the stimulus properties of *l*-cathinone may be mediated by brain dopaminergic systems and not by serotonergic neurons.

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