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Dopaminergic Mediation of a Behavioral Effect of *l*-Cathinone

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SCHECHTER, M. D. *Dopaminergic mediation of a behavioral effect of l-cathinone.* PHARMACOL BIOCHEM BEHAV **25**(2) 337–340, 1986.—Ten male rats were trained to discriminate between the stimulus properties of 0.6 mg/kg *l*-cathinone and saline in a two-lever food-motivated operant task. Once trained, rats showed a dose-dependent increase in discrimination over a dosage range of 0.15–1.2 mg/kg *l*-cathinone. Analysis of this dose-response relationship indicated an ED50 of 0.27 mg/kg. Pretreatment with 0.2 mg/kg of the specific dopamine blocking drug haloperidol increased this ED50 to 0.47 mg/kg and significantly decreased discriminative performance when co-administered with either 0.15, 0.3, or 0.6 mg/kg *l*-cathinone. Since the dose-effect curves for cathinone with and without haloperidol pre-treatment were parallel, it is suggested that *l*-cathinone, the active constituent in khat, produces its discriminative properties, in part, by mediation of dopaminergic neuronal systems.

khat Dopamine Cathinone Stimulus properties of drugs Amphetamine Haloperidol

CATHINONE, i.e., 1-phenyl-2-aminopropanone, has been established as the active psychostimulant component of the shrub *Catha edulis* [29,31]. The fresh leaves of the plant (khat) produce euphoria, excessive talkativeness, increased ability to concentrate, excitement, alteration of hunger and insomnia [7,8] and they are employed for these central stimulant effects by inhabitants of East Africa and the Arabian peninsula. These actions are reminescent of those produced by amphetamine. Indeed, cathinone is structurally similar to amphetamine and both behavioral and biochemical studies have indicated the similarity between these two agents [11–15, 20]. Furthermore, both substances suppress food intake in rodents [32] and cross-tolerance to this effect has been reported [3].

This [5, 27, 28] and one other laboratory [10] have shown that *dl*-cathinone can function as a drug capable of controlling discriminative responding in rats. Furthermore, when rats are trained to discriminate *dl*-cathinone or *d*-amphetamine from saline, they respond upon the amphetamine-appropriate lever when treated with *dl*-cathinone [22] and vice versa [28]. The discriminative stimulus properties of *d*-amphetamine have consistently been reported to be antagonized by pretreatment with the post-synaptic dopamine blocking drug haloperidol [2, 22, 23, 26] and this has led to the suggestion that the stimulus properties of *d*-amphetamine reside in dopaminergic neuronal systems [26]. In contrast, pre-treatment with 0.1 mg/kg haloperidol did not effect the discrimination of *dl*-cathinone in amphetamine-trained rats [22] and both 0.07 and 0.15 mg/kg haloperidol did not significantly attenuate discrimination of *dl*-cathinone in rats trained to discriminate a 1.0 mg/kg dose of that drug [10]. These results would suggest that the stimulus properties of *dl*-cathinone may not be identical to those of *d*-amphetamine, i.e., based upon dopaminergic mediation.

In light of the fact that it is the *l*-isomer of cathinone that is the active constituent of the khat plant [16], this isomer was recently employed to train rats to make differential discriminative responses on a two-lever food-motivated operant task and the *l*-isomer was reported to be approximately twice as potent as the *dl*-racemer and 3 times more potent than the *d*-isomer [25]. The purpose of the present study was to investigate if pretreatment with a higher dose of haloperidol (0.2 mg/kg) than formerly used [10,22] could effect the discriminative performance observed after a range of *l*-cathinone doses.

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 9W 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 [25]. There were two training phases. In the first phase, food-deprived subjects were trained to lever press on both levers for food reinforcement 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 administration, the opposite lever was activated and rats were trained on an FR1 schedule 15 min 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 [24], it was decided to modify previously employed [28] 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 select the correct lever, according to the drug condition imposed on a given day, on 18 of 20 consecutive daily sessions before it was allowed to be used for data collection.

Dose-Response Relationships to l-Cathinone

Once these animals attained the training criterion, they were tested for their sensitivity to various doses of *l*-cathinone. 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 ensure and maintain behavioral discrimination of the trained 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. On Tuesdays and Thursdays, the rats were injected IP with doses of cathinone differing from that used for initial training, i.e., 0.15, 0.3 and 1.2 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 cathinone doses was 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).

Pretreatment with Haloperidol

Test days were subsequently used to investigate the effects of pretreatment with haloperidol prior to injection of saline and each of the doses of *l*-cathinone. Haloperidol, at a dose (0.2 mg/kg) previously observed to antagonize *d*-amphetamine discrimination in a similar paradigm [26], was administered 15 min prior to the *l*-cathinone dose or saline and the rats were tested 15 min after the second injection. 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 salt of *l*-cathinone was supplied by Dr. Richard Hawks of the National Institute of Drug Abuse. This drug was dissolved in saline with doses calculated as the salt and were administered IP in a volume of 1 ml/kg 15 min prior to testing. Haloperidol (McNeil) was diluted from ampules to 0.2 mg/ml in saline.

Measurements

The lever pressed 10 times first was designated as the "selected" lever. The percentage of rats selecting the lever appropriate for the training drug was the quantal measurement of discrimination and quantal data are presented as percent correct first choice responses on the cathinone-correct lever. In addition, the number of responses on the cathinone-correct lever divided by total responses on both levers 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 [30]. The quantal data for the dose-response experiments were analyzed by the method of Litchfield and Wilcoxon [19] which employs probit vs. log-dose effects and generates ED50's and tests for parallelism. The quantitative measurements were likewise analyzed [19] and, furthermore, were compared by a Student *t*-test of means with $p<0.05$ chosen as the level of significance.

RESULTS

The attainment of the discrimination criterion (see the Method section) for the 10 rats required 25 training sessions with each of the two conditions, i.e., 0.6 mg/kg *l*-cathinone and saline, according to the pseudorandom schedule of ad-

TABLE 1

DISCRIMINATION OF *l*-CATHINONE WITH AND WITHOUT PRE-TREATMENT WITH 0.2 mg/kg HALOPERIDOL

Dose mg/kg	No. Trials	*l*-Cathinone Quantal	*l*-Cathinone Quantitative (S.D.)	*l*-Cathinone +0.2 mg/kg Haloperidol Quantal	*l*-Cathinone +0.2 mg/kg Haloperidol Quantitative (S.D.)	t	$p<$
1.2	(2)	100.0	88.8 (10.5)	75.0	72.2 (8.2)	1.75	0.11
0.6	(8;2)*	92.5	82.8 (7.3)	65.0	61.9 (16.3)	2.97	0.01
0.3	(2)	70.0	66.2 (3.1)	30.0	35.1 (18.2)	2.58	0.05
0.15	(2)	35.0	35.9 (0.1)	20.0	25.9 (3.5)	3.98	0.03
0.0 (Saline)	(8;2)†	5.6	31.5 (19.9)	10.0	16.4 (17.6)	0.51	0.31
ED_{50}		0.269	0.220	0.468	0.449		
95% conf. limit		(0.142–0.511)	(0.118–0.409)	(0.296–0.813)	(0.225–0.885)		

Parallelism:

Quantal—critical $t=2.776$ > calculated $t=0.152$

Quantitative—critical $t=2.776$ > calculated $t=0.062$

*Indicates eight maintenance trials with the training dose of *l*-cathinone without haloperidol and two test trials with co-administered haloperidol and *l*-cathinone.

†Indicates eight maintenance trials with saline administered alone and two trials with haloperidol and saline.

ministration. Once criterion was reached, maintenance sessions with 0.6 mg/kg *l*-cathinone produced 92.5% of quantal responding upon the cathinone-correct lever, whereas saline administration produced 5.6% responding upon this lever (or 94.4% quantal responses upon the saline-correct lever) as presented in Table 1. The administration of 1.2 mg/kg *l*-cathinone produced 100% quantal responding upon the cathinone-lever and decreasing doses of cathinone resulted in decreasing discriminative performance. Analysis of the quantal dose-response relationship [19] resulted in an ED50 (with 95% confidence limits) of 0.269 (0.142–0.511) mg/kg. Likewise, decreasing doses of cathinone produced a progressively decreasing dose-response relationship for the quantitative measurement and an ED50 of 0.220 (0.118–0.409) mg/kg.

Administration of 0.2 mg/kg haloperidol, 15 min prior to cathinone injection, significantly decreased the discrimination of the 0.6, 0.3 and 0.15 mg/kg dose of cathinone. The quantal dose-response curve generated from the co-administration of 0.2 mg/kg haloperidol and the various doses of cathinone indicated an ED50 of 0.468 mg/kg, whereas the quantitative dose-response curve had an ED50=0.449 mg/kg. Comparison of the quantal and quantitative cathinone dose-response curve with and without pretreatment of haloperidol [19] indicated that they were parallel within 95% statistical limits.

DISCUSSION

The present report indicates that *l*-cathinone, like its racemic mixture [5, 10, 27, 28] can function as a drug to control discrimination behavior in the rat, and that this discrimination is dose-responsive. In addition, pretreatment with 0.2 mg/kg haloperidol was observed to significantly attenuate cathinone discrimination. This confirms and expands one previous report [25] and is in conflict with one other report [10] that indicated that lower doses of haloperidol, i.e., 0.07 and 0.15 mg/kg, did not significantly decrease discrimination of 1.0 mg/kg *dl*-cathinone. Unfortunately, this latter report did not use statistical measurements but, rather, an 80% quantal criterion as the measurement to indicate cathinone discrimination. The present study employed a quantitative measurement (see the Method section and [30]) that allows for the application of parametric statistical analysis in addition to the "all-or-none" quantal measurement. Analysis of data after co-administration of 0.2 mg/kg haloperidol and either 0.6, 0.3 or 0.15 mg/kg *l*-cathinone indicates a significant decrease in cathinone discrimination. The ED50's derived from data on quantal and quantitative measurements were seen to be in close agreement as has been reported to occur with amphetamine and cocaine in a similar behavioral procedure [30].

A large number of studies have demonstrated that amphetamine is capable of controlling discriminative responding in a two-choice discrimination task [1, 9, 10, 22, 26, 30] and there is considerable evidence that dopamine is involved in the mediation of the amphetamine related stimuli which control this choice behavior. Thus, haloperidol has repeatedly been shown to block amphetamine discrimination in the rat [2, 9, 21, 23, 26]. The present study indicates that haloperidol, at 0.2 mg/kg, also can antagonize the *l*-cathinone discriminative cue over a range of cathinone doses and the parallelism between the dose-response curves with and without haloperidol indicates that the same mechanism and/or receptors are involved [18]. These data, thus, suggest that dopaminergic systems are involved in the discriminative properties of *l*-cathinone but, because of the larger doses of haloperidol needed to produce this antagonism, this involvement does not appear to be as great as that seen in the discriminative properties of amphetamine. In light of other

recent reports that indicate either failure [10,22] or only partial antagonism [6] of discrimination of *dl*-cathinone with haloperidol, the mediation of the discriminative properties of cathinone may involve other neurotransmitter systems. Indeed, cathinone has been reported to have affinity for serotonin receptors in the periphery [4] and to produce release of serotonin from brain tissue [17]. This would suggest that the discriminative properties of cathinone, unlike amphetamine, may be dependent upon both dopaminergic and serotonergic neuronal systems.

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