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Induction of and Recovery from Tolerance to the Discriminative Stimulus Properties of *l*-Cathinone

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SCHECHTER, M. D. *Induction of and recovery from tolerance to the discriminative stimulus properties of l-cathinone.* PHARMACOL BIOCHEM BEHAV **25**(1) 13–16, 1986.—Rats previously trained to discriminate between 0.6 mg/kg *l*-cathinone and saline in a two-lever, food-motivated operant task were administered *l*-cathinone at the same dose, every 8 hours for 10 days. Discrimination testing during this chronic administration phase of experimentation indicated that the animals' ability to discriminate both 0.3 and 0.6 mg/kg *l*-cathinone decreased when compared to their discriminative ability prior to chronic administration. In contrast, discrimination of the non-drug state, i.e., saline, was not affected. Comparison of dose-response curves prior to and during chronic cathinone administration indicated a 3–4 fold shift to the right for the latter curve. Continued testing after termination of chronic treatment resulted in a return to pre-chronic discriminative performance by the fifteenth day after cessation. These results indicate that tolerance to the discriminative effects of *l*-cathinone can be produced within 10 days of chronic administration and recovery from this observed tolerance occurs within 15 days of cessation of chronic administration.

Cathinone Chronic administration Drug discrimination Tolerance Dopamine

l-CATHINONE is the psychoactive alkaloid found in the leaves of *Catha edulis* (Khat) which is chewed for its central stimulatory effects by the inhabitants of several eastern African countries [9]. Both the chemical structure and behavioral effects of cathinone are similar to those of amphetamine [4, 9, 17] but, unlike amphetamine, the possibility of development of tolerance to Khat effects in human abusers has been excluded on the basis of quantitative effects, i.e., the physical limits on the amount of Khat leaves that can be chewed [7]. Nevertheless, the physiological responses, e.g., increased diastolic blood pressure and respiratory rate, to Khat chewing were found to be significantly less in chronic consumers than in naive users [13]. In addition, tolerance to the anorectic effect of cathinone in rats has been reported [4,20].

A behavioral paradigm which is particularly suited for assessing the "subjective" effects of psychoactive drugs is the drug discrimination procedure. This sensitive, reliable and specific paradigm not only can be employed to test the similarity or dissimilarity between psychoactive drugs, but it also can be used to investigate the production of tolerance [1, 3, 6, 8, 11, 12, 16, 19]. Cathinone is capable of serving as the discriminative stimulus to permit rats to make differential responses in an operant task [5, 15, 17]. The purpose of the present study was to train rats to discriminate *l*-cathinone and, then, to attempt to produce tolerance by chronic administration of *l*-cathinone, and finally, to observe the possibility of recovery from tolerance by continued testing of the rats after the cessation period of chronic treatment.

METHOD

Subjects

The subjects were nine male ARS/Sprague-Dawley rats weighing 670–800 g at beginning of the study. The animals were housed in individual cages, and their weights were maintained at approximately 80–85% of the expected free-feeding weights by partial food deprivation. Water was continuously available in the home cages which were kept in a temperature-controlled (20–22°C) room with a daily cycle of 12 hr (0600–1800) light and 12 hr dark.

Apparatus

The apparatus consisted of eight identical two-lever operant chambers (Lafayette Instruments Corp., Lafayette, IN) housed within individual sound-attenuating cubicles equipped with an exhaust fan and a 9 W house-light. Each chamber contained two operant levers located 7 cm apart and 7 cm above the grid floor. A food pellet receptacle was mounted 2 cm above the grid floor and equidistant between the 2 levers. Solid-state programming equipment (Med Assoc., E. Fairfield, VT) was used to control experimental contingencies and record responses, and was located in an adjacent room.

Discriminative Training

The animals used in this study were the same nine rats previously trained to discriminate *l*-cathinone from saline;

their training has already been described in detail [14]. In brief, these animals were first trained to press one of the two levers for food (45 mg Noyes pellets) reinforcement on a fixed ratio 10 (FR 10) schedule. Throughout this training, the animals received daily intraperitoneal (IP) injections of saline 15 min prior to being placed into the operant chamber. The animals were then trained to discriminate an equal volume (1 ml/kg) of saline containing 0.6 mg/kg *l*-cathinone administrated IP. For five rats, responding on the left lever was reinforced after administration of the drug, while for the other four rats responding on the right lever was reinforced following drug administration. Responses on the opposite lever were reinforced after saline administration. The rats were then trained 5 days per week with reinforcements in a pseudo-random sequence. Thus, in each two-week period there were five days with drug lever (D) and five days with saline lever (S) correct. The pattern was D,S,S,D,D; S,D,D,S,S. The rats had to respond on the appropriate lever to receive food reinforcement. Which lever was correct was dependent upon whether the training drug or saline had been administered prior to the start of the session. Responses upon the inappropriate lever were recorded, but they produced no programmed consequence. Training criterion was reached when the animals selected the appropriate lever, according to the drug or non-drug (saline) state imposed, on two sets of nine out of ten consecutive sessions.

Pre-Chronic Dose-Response Determinations

After the rats had attained the discriminative training criterion, sessions of 15 min duration with alternating administrations of 0.6 mg/kg *l*-cathinone and saline were continued on Mondays, Wednesdays, and Fridays. This procedure was meant to ensure and maintain discrimination to the training drug conditions. On Tuesdays and Thursdays, doses of *l*-cathinone (0.15, 0.3 and 1.2 mg/kg) different than the 0.6 mg/kg training dose were administered IP 15 min before placing the animals into the operant chamber. Each dose of *l*-cathinone was tested on two occasions, each preceded by one 0.6 mg/kg cathinone and one saline maintenance session. During these test sessions, the animals were allowed to respond, in extinction, until 10 responses were made on either lever, and were then returned to their home cages. 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. In addition, the total number of responses on both levers, made before 10 responses on either lever were counted, constitutes the quantitative measurement, i.e., the number of responses on the "cathinone correct" lever, divided by total responses made prior to 10 responses (including the 10 on the cathinone lever), times 100. The quantal data for the dose-response experiments were analyzed by the method of Litchfield and Wilcoxon [10] which employs probits vs. log-dose effects and generates ED50's and tests for parallelism. Verification of analysis was made with a TRS-80 computer using published computer programs [18]. The quantitative measurements were compared by a Student *t*-test of means with $p>0.05$ chosen as the level of significance.

Chronic Regimen and Testing Schedule

After the dose-response experiments were conducted, all rats were injected IP with 0.6 mg/kg *l*-cathinone every 8 hours, at 0900, 1700, and 0100 hr, for 10 consecutive days. During this regimen of chronic administration, the rats were tested, in extinction, with either saline, 0.3,0.6 or 1.2 mg/kg *l*-cathinone according to the following schedule:

Day	Time	Treatment
2	1600	0.6 mg/kg *l*-cathinone
3	0800	saline
3	1600	1.2 mg/kg *l*-cathinone
4	1600	0.6 mg/kg *l*-cathinone
5	1600	0.3 mg/kg *l*-cathinone
6	0800	saline
6	1600	0.6 mg/kg *l*-cathinone
7	1600	0.3 mg/kg *l*-cathinone
8	1600	0.6 mg/kg *l*-cathinone
9	0800	saline
9	1600	1.2 mg/kg *l*-cathinone
10	1600	0.6 mg/kg *l*-cathinone

Prior to each test session, the food receptacle was "baited" with a food pellet but responding did not result in delivery of an additional pellet and the rats were immediately removed upon making 10 responses on either lever. According to the treatment schedule, 0.6 mg/kg *l*-cathinone was tested on every second day, saline was tested on every third day and the two other doses of *l*-cathinone were counterbalanced within the 10 days of testing.

Post-Chronic Discrimination Testing

After 10 days of chronic *l*-cathinone administration and testing according to the treatment schedule, non-contingent administration of 0.6 mg/kg *l*-cathinone was terminated. For the remainder of the study, the rats were continued in test sessions, in extinction, repeating the same schedule, until discrimination of 0.6 mg/kg *l*-cathinone was observed to approximate pre-chronic levels.

RESULTS

The results of discriminative responding expressed in terms of quantal and quantitative measurements (see the Method section) prior to, during, and after chronic administration of 0.6 mg/kg *l*-cathinone, appear in Table 1. The dose-response data prior to chronic administration (I. Pre-Chronic) indicates errorless quantal discrimination with the training conditions and a decreased discriminative performance with decreasing *l*-cathinone doses. Analysis of the dose-response curve [10] yields an ED50 (with 95% confidence limits) of quantal data=0.111 (0.059–0.209) mg/kg and a similar ED50 of 0.127 (0.068–0.236) mg/kg for the quantitative measurements.

Discriminative testing during the chronic regimen of treatment is presented as "II. Chronic" in Table 1. Saline discrimination, tested at 0800 on days 3, 6, and 9, remained errorless, as did discrimination of 1.2 mg/kg *l*-cathinone on days 3 and 9. The quantal discrimination of 0.6 mg/kg *l*-cathinone, however, decreased to 66.7% by day 10 and the discrimination of 0.3 mg/kg *l*-cathinone was at 22.2% on both the 5th and 7th day of the chronic regimen. When the quantitative measurements for 0.6 mg/kg *l*-cathinone on days 4–10 of the chronic regimen or 0.3 mg/kg l-cathinone on days 5

TABLE 1

DISCRIMINATIVE RESPONDING PRIOR TO, DURING AND AFTER CHRONIC ADMINISTRATION OF *l*-CATHINONE

		I. Pre-Chronic	
Treatment [# trials]	Dose (mg/kg)	Quantal	Quantitative (SD)
Saline [4]	—	0.0	10.6 (2.9)
l-cathinone [2]	1.2	100.0	98.9 (0.2)
[4]	0.6	100.0	92.6 (3.7)
[2]	0.3	94.4	89.6 (1.8)
[2]	0.15	44.4	48.7 (7.4)
ED50: (95% confidence)		0.111 (0.059–0.209)	0.127 (0.068–0.236)

		II. Chronic		III. Post-Chronic	
Treatment (day)	Dose (mg/kg)	Quantal	Quantitative	Quantal	Quantitative
Saline (3)	0.0	0.0	5.3	0.0	9.1
(6)		0.0	6.3	0.0	9.1
(9)		0.0	5.3	0.0	12.6
(13)				0.0	13.5
l-cathinone (2)	0.6	100.0	94.7	88.9	81.8
(4)		77.8	65.6	77.8	68.5
(6)		100.0	79.7	88.9	77.4
(8)		77.8	71.2	100.0	80.4
(10)		66.7	65.8	88.9	71.3
(14)		—		88.9	85.6
l-cathinone (5)	0.3	22.2	31.4	33.3	43.4
(7)		22.2	31.9	66.7	61.0
(15)		—		88.9	79.5
l-cathinone (3)	1.2	100.0	95.8	100.0	91.8
(9)		100.0	97.8	100.0	92.3
ED50		0.431 (0.293–0.635)	0.407 (0.278–0.598)	0.268 (0.166–0.432)	0.267 (0.116–0.546)

and 7 are compared to their respective pre-chronic quantitative measurements there is a significant decrease in discrimination, at $p>0.002$ and $p>0.0003$, respectively, during the chronic test period. Analysis of the dose-response curve during chronic *l*-cathinone administration generates an ED50=0.431 mg/kg for quantal and 0.407 mg/kg for quantitative data.

Following termination of chronic *l*-cathinone, the discrimination of 0.6 mg/kg returned to 100% by the 8th day (III. Post-Chronic, Table 1) and continued at 88.9% for the remainder of the experiment; this reflects one of the 9 rats choosing the saline appropriate lever on days 10 and 14. The discrimination of 0.3 mg/kg *l*-cathinone steadily increased from day 5 to day 7 post-chronic administration and reached 88.9% during the second schedule of testing post-chronically, i.e., day 15. The post-chronic ED50 was found to be 0.268 mg/kg and the 95% confidence range overlaps with the range calculated for the pre-chronic data.

DISCUSSION

Tolerance is generally defined either as a decreased intensity of a response to the same amount of drug or as the phenomenon that a greater amount of drug is required to obtain a response of the intensity which is similar to that of the original response to the drug. Tolerance, thus, was observed to develop to the discriminative stimulus properties of *l*-cathinone after 10 days of chronic injection with *l*-cathinone (0.6 mg/kg every 8 hr). These results are in agreement with one previous report that indicated that chronic administration of cathinone produces a decrease in the daily amount of milk consumed by rats, whereas after continued adminstrations the rats return to pre-chronic levels of consumption [4]. Furthermore, the tolerance developed to the suppressant effects of cathinone on milk drinking after 7 days, as evidenced by a shift to the right of the dose-response curve, and returned to pre-session values in 16 days; a time-course similar to that observed in the present study.

Chronic administration of *l*-cathinone produced a 3–4 fold shift to the right of the discriminative dose-response curve for cathinone. This magnitude of tolerance was similar to that seen in studies of tolerance to cocaine in a similar behav-

ioral paradigm [19]. The possible development of tolerance in a drug discrimination paradigm has been critiqued by Colpaert [2] in those studies in which rats receive non-contingent exposure to progressively increasing doses of the training drug (e.g., [1]). This treatment regimen would theoretically have the effect of resetting the training "set point" which was originally set by the training drug dose, i.e., exposure to doses higher than the training dose would have the same effect as simply increasing the training dose. In the present study, tolerance developed to chronic administration of the same dose (0.6 mg/kg) of *l*-cathinone used to train the rats.

The present procedure, however, may produce another shortcoming, viz., the measurement of tolerance based upon extinction data, i.e., long-term testing without continued training. Thus, non-reinforcement of correct-choice responding over the 26 days of the present study may have led to extinction of the discriminative stimulus produced by initial training. However, inspection of the results of interspersed sessions with saline discrimination indicated that, at least for the non-drug state, there was no loss of discriminative performance. Furthermore, when the chronic administration of cathinone was terminated, the baseline sensitivity spontaneously recovered without retraining the rats to attend to a smaller stimulus value.

Although the cause of cathinone tolerance was not explored in this investigation, the present results do indicate that tolerance occurs to the discriminative stimulus properties of this drug. The possibility of metabolic tolerance as caused by enzyme induction to repeated drug administrations cannot be ruled out. However, since cathinone discriminative performance appears to be mediated by brain dopamine [14], one may speculate that cathinone tolerance may result through an alteration of dopamine function.

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