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Dopaminergic Nature of Acute Cathine Tolerance

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SCHECHTER, M. D. *Dopaminergic nature of acute cathine tolerance*. PHARMACOL BIOCHEM BEHAV **36**(4) 817–820, 1990.—Cathine is a psychoactive constituent in the leaves of the Khat shrub which are habitually ingested for their stimulatory effects in many parts of the world. Rats were trained to discriminate the stimulus effect of intraperitoneally administered 4.8 mg/kg *d*-cathine and, once trained, administration of another Khat constituent, cathinone, was shown to produce cathine-like effects. This generalization to cathinone was dose-responsive when testing occurred 24 hr after vehicle administration, whereas prior administration of cathine resulted in a diminished discriminative response to subsequent cathinone administration possibly as a result of the development of acute tolerance. CGS 10746B, a compound that blocks presynaptic release of dopamine, significantly decreased rats' ability to discriminate cathine when it was administered 25 min prior to cathine testing and it reversed the acute tolerance observed when cathine was tested 24 hr after cathine administration. These results indicate that a previously reported acute tolerance effect to cathine after cathinone administration in cathinone-trained rats appears to be symmetrical in that there is acute tolerance to cathinone after cathine in these cathine-trained rats. The results with CGS 10746B would suggest that both the cathine-induced discriminative cue and cathine's ability to produce acute tolerance are mediated by presynaptic dopamine release.

Drug discrimination Cathine Khat Dopamine Cathinone CGS 10746B

THE leaves of *Catha edulis*, a plant native from Arabia through eastern Africa, are the source of Khat which has been used as a central nervous system stimulant from a time thought to antedate that of coffee (7). Today, several million people in these regions are habitual Khat chewers; undoubtedly, for the pharmacological actions produced by the presence of the two psychoactive phenylalkylamines cathinone and cathine (14). Small amounts of cathine (norpseudoephedrine) are found in fresh Khat leaves, whereas the main constituent of the leaf is cathinone (11). After the leaves of the shrub are picked and subsequently dried, cathinone is converted into cathine with the latter substance being less potent (21). Cross-tolerance between the effects of these two "khatamines" and that of amphetamine has been observed in numerous laboratories (2, 3, 12, 23). In addition, these Khat ingredients have received considerable research attention and both have been shown to resemble amphetamine in their actions upon the central nervous system including release, and blockade of reuptake, of dopamine (9, 10, 22, 24) their ability to induce hyperactivity in rodents (6, 8, 25) and being capable of serving as discriminative stimuli (5, 16–18, 20). In rats trained to discriminate the interoceptive cues produced by cathinone, the administration of cathine has been observed to produce cathinone-like responding; an ability known as "generalization." In addition, acute tolerance, i.e., tolerance after a single dose, appears to occur when cathine is tested 24 hr after cathinone administration (18).

The purpose of the present study was to investigate if cathine, a drug that has only recently been shown to be capable of controlling discriminative stimulus behavior (16), generalizes to cathinone, if this generalization is affected by the phenomenon of acute tolerance and, lastly, if the cathine cue and/or its production of tolerance is mediated by dopaminergic mechanisms. In regard to this last point, the benzothiadiazepine CGS 10746B was used since this compound has been shown to decrease dopamine release without either changing dopamine metabolism or occupying dopaminergic receptors (1). Recent evidence indicated that CGS 10746B antagonizes, in a dose-response manner, the ability of rats to discriminate *d*-amphetamine (19), as well as cathinone (18).

METHOD

Subjects and Discrimination Training Procedure

Twelve male Sprague-Dawley rats purchased from Zivic-Miller Laboratories (Allison Park, PA) were maintained at 80–85% of their free-feeding body weights and provided with water ad lib in their individual home cages. The apparatus used in training consisted of standard operant chambers (Lafayette Instruments Corp., Lafayette, IN) each containing two levers and provided with a food dispenser to deliver the food reinforcement, i.e., 45 mg food pellets (P. J. Noyes Co., Lancaster, NH).

The shaping to lever-press and discrimination training has been previously described in detail (16). Briefly, the animals were trained to press one lever 15 min following the intraperitoneal (IP) administration of vehicle (distilled water) at a volume of 1 ml/kg. The initial FR1 reinforcement schedule was gradually increased, over a 5-day period, until an FR10 reinforcement schedule was attained. This procedure was then repeated with the other lever being reinforced following a similar volume of water containing 4.8 mg/ml of *d*(+)-cathine hydrochloride (NIDA) with the weight

TABLE 1

DISCRIMINATIVE GENERALIZATION TO 0.2–1.2 mg/kg CATHINONE 24 HR AFTER VEHICLE OR CATHINE ADMINISTRATION IN RATS (n = 12) TRAINED TO DISCRIMINATE 4.8 mg/kg CATHINE FROM ITS VEHICLE

	Discrimination Performance After:			
	Vehicle		Cathine	
Dose (mg/kg)	Quantal	Quantitative (S.D.)	Quantal	Quantitative (S.D.)
1.2	95.8	79.6 (2.2)	95.8	77.5 (5.2)
0.8	83.3	70.5 (0.5)	66.7	64.4 (15.7)
0.4	75.0	73.1 (6.9)	20.8	29.0 (4.0)*
0.2	20.8	29.9 (0.9)		ND
ED_{50} (mg/kg) (95% C.L.)	0.32 (0.23–0.44)		0.60 (0.50–0.71)	

ND: not determined.
*Significantly lower than quantitative measurement after vehicle; $p<0.01$ (t-test).

calculated as the salt. Fifteen min after IP administration, the rats were required to press the second of the two identical levers on an FR1 schedule and the reinforcement requirement was gradually increased, over 3 days, to an FR10. Once lever-pressing behavior was established on both levers, a biweekly repeating injection order was employed: V,D,D,V,V; D,V,V,D,D, where V = vehicle, D = drug, i.e., 4.8 mg/kg cathine. For each rat, responses on any given day were considered correct if the first lever to accumulate 10 presses was state-appropriate, i.e., the cathine lever after cathine administration and the vehicle lever after vehicle administration. Training was continued, on Mondays to Fridays, until all rats attained the training criterion of 16 correct first lever selections out of 20 consecutive sessions.

Generalization to Various Doses of Cathinone

After criterion performance was established, every second day was employed as a maintenance day in which the animals were administered, on an alternating basis, either the training (4.8 mg/kg) dose of cathine or its vehicle and required to select the appropriate lever for which they received 40 food pellets on the FR10 schedule of reinforcement. On interspersed days, the animals were tested with novel drugs in extinction, i.e., the session was terminated without reinforcement immediately after ten responses accumulated on either of the two levers. Each animal received all treatments and the treatment order was randomized with the doses of cathinone selected as 0.2, 0.4, 0.8 and 1.2 mg/kg; a range of doses previously shown to produce dose-responsive effects in animals trained to discriminate cathinone (18). Each dose was tested four times; on two occasions 24 hr after cathine maintenance and in two trials 24 hr following vehicle maintenance.

Antagonism With CGS 10746B

On subsequent test sessions, CGS 10746B was administered (IP) 10 min prior to the administration of either vehicle or 4.8 mg/kg cathine and the rats were tested 15 min following the second injection. Thus, the time interval between injection of the trained drug or its vehicle and testing (15 min) was kept constant, whereas the CGS 10746B was employed at a time prior to testing similar to that previously shown to allow for its maximal effect upon dopamine release (1). As in the cathinone generalization experiments (above), the animals were immediately removed and returned to their home cages, without receiving reinforcement, upon accumulating 10 responses on either of the two levers. Doses of CGS 10746B used were 10 and 20 mg/kg and each of the doses was administered prior to vehicle and tested on two occasions, and prior to cathine and, likewise, tested on two occasions. In addition, each of these two doses of CGS 10746B was administered prior to cathine and, 24 hr later, cathine was again administered and tested. On other test days, cathine was tested 24 hr after either vehicle maintenance or cathine maintenance sessions.

Measurements and Statistical Analysis

The percentage of rats selecting the lever appropriate for cathine was the quantal measure of discrimination and is presented as percentage of rats making the first choice selection on the cathine-correct lever (an all-or-none effect). The dose-response relationship observed in the generalization experiments with cathinone 24 hr after vehicle maintenance and 24 hr after cathine maintenance was subjected to analysis by the procedure of Litchfield and Wilcoxon (13). This method employs log-dose vs. probit measurements and allows for generation of ED_{50} values with 95% confidence limits.

The quantitative measurement used represents the total number of lever presses accumulated on both levers prior to the completion of 10 presses on either lever, i.e., the number of presses on the cathine-correct lever divided by the total responses made on both levers (including the 10 responses on the cathine lever), times 100. This measurement was derived each session and it was included in order to analyze data on both levers as to be able to incorporate responses upon the "unselected" lever in the statistical analysis. Quantitative measurements were compared by application of Student's t-test with $p<0.05$ set as the level of significance.

RESULTS

As previously reported, rats were capable of employing the training dose of 4.8 mg/kg $d(+)$-cathine in order to make differential responses in a two-lever operant task. Discriminability of cathine vs. its vehicle continued in all twelve animals throughout the experiment as indicated by their selected lever performance on intervening maintenance days. The ability of cathine-trained rats to generalize to cathinone was observed to be dose-dependent but different dose-response curves were generated depending upon the prior day's (maintenance) treatment (Table 1). Thus, for

TABLE 2

EFFECT OF CGS 10746B ON (A) 4.8 mg/kg CATHINE AND VEHICLE DISCRIMINATION WHEN ADMINISTERED 25 MIN PRIOR TO TESTING, AND (B) CATHINE DISCRIMINATION 24 HR AFTER CATHINE ADMINISTRATION

(A) CGS 10746B

Dose (mg/kg)	+ Vehicle		+ Cathine	
	Quantal	Quantitative (S.D.)	Quantal	Quantitative (S.D.)
10	0.0	4.8 (0.0)	37.5	46.6 (9.9)
20	0.0	2.8 (0.6)	29.2	35.8 (4.4)

(B) Treatment(s):

Day 1	Day 2	Quantal	Quantitative (S.D.)
vehicle	cathine	100.0	83.4 (6.0)
cathine	cathine	59.1	59.2 (12.4)
10 mg/kg CGS + cathine	cathine	83.3	74.0 (8.8)
20 mg/kg CGS + cathine	cathine	95.5	79.6 (3.6)*

Intervening Maintenance Days

	Quantal	Quantitative (S.D.)
vehicle	4.2	9.5 (9.1)
cathine	86.7	72.6 (6.1)

*Significantly greater than the quantitative measurement of cathine discrimination (59.2 ± 12.4) 24 hr after cathine administration alone ($p<0.05$; t-test).

example, in the results representing cathinone tested 24 hr after vehicle administration, 1.2 mg/kg cathinone resulted in 23 of 24 trials (12 rats × 2 sessions at each dose) in which the cathine-appropriate lever was first pressed 10 times; this allows for a 95.8% quantal measurement. Decreasing the dose of cathinone resulted in decreased quantal measures and yielded an ED_{50} value (with 95% confidence limits) of 0.32 (0.23–0.44) mg/kg. When the three highest doses (0.4–1.2 mg/kg) of cathinone were tested on two occasions each 24 hr after cathine administration, a steeper dose-response curve was generated with an ED_{50} value (95% confidence limits) equal to 0.60 (0.50–0.71) mg/kg obtained. The lowest dose (0.2 mg/kg) cathinone was not determined as the 0.4 mg/kg after cathine maintenance produced a quantal measurement of 20.8%. Relative to performance on the day following vehicle administration, significantly more animals choose the cathine-appropriate lever following 0.4 mg/kg cathinone than when it was tested on the day following cathine administration.

Pretreatment with two doses of CGS 10746B 10 min prior to the administration of the vehicle produced exclusively vehicle-like responding when the rats were tested 15 min after vehicle injection (Table 2A). In contrast, CGS 10746B pretreatment at both 10 and 20 mg/kg antagonized the cathine discriminative cue as shown by a reduction of the maintenance day cathinone performance from a quantal measurement of 86.7 to 37.5 and 29.2%, respectively.

When the training dose of cathine was tested 24 hr after vehicle administration 100% of responses were made on the cathine-appropriate lever (Table 2B). In contrast, 24 hr after cathine administration, testing of cathine resulted in 59.1% of cathine-lever selections. This indicates that the previous day's maintenance treatment had a significant effect upon cathine discrimination. However, when either 10 or 20 mg/kg CGS 10746B was administered 10 min prior to cathine on the first day, cathine discrimination on the following day was seen not to be significantly affected. Thus, when 20 mg/kg CGS 10746B was administered prior to the training dose of cathine on day one and this was followed (on day two) with a cathine test session, the results indicate a quantitative measurement that is significantly greater than when cathine was tested 24 hr after cathine without pretreatment with CGS 10746B. Testing of higher doses of CGS 10746B in combination with cathine was precluded by the appearance of behavioral disruption.

DISCUSSION

The results indicate that rats were more sensitive to cathinone test doses 24 hr after vehicle administration (on maintenance days) than on test days following cathine administration. Comparison of the dose-response curves after cathine and vehicle maintenance sessions indicate a two-fold lower ED_{50} value, and a significantly greater discrimination of 0.4 mg/kg cathinone, following vehicle than following cathine. Previous work from this laboratory has indicated that, in animals trained to discriminate either cathinone or amphetamine, the discriminability of cathine was reduced when tested 24 hr after either drug (18). In cathine-trained animals, the discrimination of cathine is also reduced if preceded by cathine administration either in maintenance training or by noncontingent administration (16). The present study extends these previous reports to indicate that the generalization from cathine to cathinone is also affected by the previous day's drug treatment.

These observations may be explained by the possibility that acute tolerance develops to all of these drugs. Thus, cathinone, cathine and amphetamine are all indirect-acting dopamine agonists, i.e., they all release dopamine (9, 10, 22, 24) and central dopamine might remain partially depleted for 24 hr after their administration. Thereby, when cathinone is tested in generalization experiments (Table 1), or when cathine is tested (Table 2B), after cathine administration, lessened discriminative performance may be seen in rats who have been trained to discriminate the dopaminergically mediated cathine discriminative cue (16). Indeed, tolerance and cross-tolerance between cathinone and cathine have been previously reported to occur by numerous investigators (2, 3, 12, 23).

The mediation of central dopaminergic neurons in cathine discrimination has previously been suggested by the ability of haloperidol pretreatment to attenuate rats' ability to discriminate it, whereas pretreatment with the solely peripheral active agent domperidone was shown to be ineffective in this regard (16). The present observation that CGS 10764B pretreatment significantly decreased animals' ability to discriminate cathine lends further evidence for the mediation of dopaminergic neurons in the interoceptive cueing properties of cathine. In addition, the CGS 10746B-induced reversal of the discriminative deficits with cathine following cathine administration would indicate that the acute tolerance that is observed may also be dopaminergically mediated.

In the human user, tolerance to the physiological effects produced by the psychoactive substances found in Khat leaves has been shown to develop as to elevated systolic blood pressure, increased respiratory rate and body temperature (15) and to the initial insomniac effects (14). However, in actual use, tolerance to the chewing of Khat leaves is difficult to measure as a result of the physical limits on the amount of leaves that can be chewed, i.e., the physical impossibility of increased leaf chewing beyond a "full" limit (7). Nevertheless, the habitual use of Khat may result in chronic hypertension, spermatorrhea, irritative disorders of the upper gastrointestinal tract (11), as well as acute psychosis (4). Interestingly, this most recent report of a psychiatric disturbance after Khat leaf ingestion was successfully treated with a dopaminergically active (antipsychotic) drug (4).

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