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Direct Microinjection of Cathinone Into the Rat Brain Produces Discriminative Stimuli

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SCHECHTER, M. D., J. B. SCHECHTER AND D. J. CALCAGNETTI. *Direct microinjection of cathinone into the rat brain produces discriminative stimuli.* PHARMACOL BIOCHEM BEHAV **42**(4) 619–623, 1992. – Rats were trained to discriminate IP administration of 800 μg/kg cathinone using a food-motivated, two-lever discrimination procedure. Following training, 800 μg/kg cathinone discrimination was produced (generalized) by lower cathinone doses in a dose-responsive manner after IP administration; an ED_{50} value of 330 μg/kg was calculated. Subsequently, guide cannulae were implanted into the lateral ventricle and bilaterally into the nucleus accumbens. After recovery, injections were made via cannulae that extended 0.5 mm past the tip of the guide cannulae. ICV administration of 256 μg cathinone/rat produced discriminative responding on the cathinone-appropriate lever to the same degree as did the peripherally administered training dose of cathinone. Decreasing ICV doses produced decreased discriminative performance and allowed the calculation of an ED_{50} value of 90.5 μg. Likewise, administration of 64 μg cathinone/nucleus accumbens (for a total of 128 μg/rat) substituted for the IP training dose of cathinone. These results evidence the central mediation of the cathinone-induced discriminative stimulus cue and show that administration of cathinone into the nucleus accumbens is sufficient to produce these stimuli. Thus, these data suggest that receptors in the nucleus accumbens are important for the discrimination of this psychostimulant.

Cathinone Discrimination stimulus Intracerebroventricular Nucleus accumbens Generalization Rat

ALTHOUGH the behavioral paradigm that employs drug-induced stimulus cues to allow for discriminative responding has been evidenced to be a specific, stable, and highly reproducible technique (6), and has resulted in over 1,100 publications from 1951–1987 (25), there is a paucity of reports that employ cerebral microinjection of the drug to test its discriminability [e.g., (17,19,28,29)]. Indeed, it has been a generation since one of us (M.D.S.) reported the transfer of state-dependent control of discriminative behavior between a subcutaneously and intraventricularly administered drug (20) These (few) studies attempt to identify the site of action of centrally injected drugs to evidence the fact that the peripherally trained discriminative stimulus cue(s) can be replicated after the same drug is administered centrally and, as Wise (27) termed it: " . . . know the minimal behaviourally significant concentration at the critical receptor."

Cathinone is a psychostimulatory compound found in the leaves of Khat, a shrub native to Arabia and eastern Africa. The subjective effects of Khat chewing closely resemble the effects of amphetamine and include euphoria, improved intellectual efficiency, and alertness (15). This relationship to amphetamine is not surprising because of the close similarity between the chemical structures of cathinone and amphetamine, that is, the only difference between them is that the two hydrogens on the β carbon of the amphetamine side chain are substituted by oxygen in cathinone. This commonality has allowed a large number of studies to show that these drugs produce similar effects in laboratory animals [see reviews: (11,12)]. Systemic administration of cathinone has been found to serve as a discriminative stimulus in rats both in this (23) and numerous other laboratories (5,8,10). Once a drug discrimination is acquired, drugs other than those used in training can be substituted for the trained drug to assess the degree of generalization between compounds. When tested in this manner, cross-generalization has been shown to occur between cathinone and amphetamine (7,9,23,24). Indeed, the only differences in cathinone and amphetamine discrimination appear to be temporal with cathinone having a shorter duration (21). Centrally administered *d*-amphetamine has been shown to not only substitute in animals trained to discriminate it after peripheral administration (17) but this route has also allowed amphetamine to produce conditioned place prefer-

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ence (1,3,4). A recent study in this laboratory (2) evidenced the ability of ICV-administered cathinone to, likewise, produce a conditioned place preference. Thus, the scientific evidence is complete, that is, cathinone and amphetamine both produce discriminative stimuli after peripheral administration and they are cross-generalized, rats that are peripherally trained with amphetamine generalize to centrally administered amphetamine, and both drugs produce conditioned place preference after ICV administration. It is the purpose of this series of experiments to "complete this picture" by investigating if animals trained to discriminate the interoceptive stimuli produced by peripherally administered cathinone generalize to ICV- and/or intraaccumbens-administered cathinone.

METHOD

Subjects, Discriminative Training, and Measurements

The 17 male Sprague-Dawley rats used in the present experiments were employed in a previous study (22) in which they were trained to discriminate IP-administered 800 μg/kg *l*-cathinone from its vehicle in a two-lever food-motivated operant task. Maintenance of the cathinone/vehicle discrimination was ensured by continuation of training sessions throughout dose-response and generalization tests, that is, at least one cathinone maintenance session and one vehicle maintenance session preceded each test session. Generalization test sessions were identical to training sessions except that after a challenge with any dose or route different than 800 μg/kg cathinone IP rats were immediately removed from the operant chamber upon making 10 responses on either lever (without receiving reinforcement). The lever upon which the rat first totaled 10 responses was regarded as the "selected" lever. The number of animals selecting the cathinone-correct lever constitutes the quantal discriminative measurement. Unlike the all-or-none quantal measurement, a quantitative measurement was also used to allow for responses on both the selected and unselected levers to be considered. This provides a relative measure of the magnitude, as well as the direction, of lever performance and the quantitative measurement was derived by dividing the number of responses on the cathinone lever by the total number of responses on both the cathinone and vehicle lever at the time that 10 responses were accumulated on either single lever. This fraction is expressed as a percentage and the results of each of two trials allows for a mean and standard deviation of the mean to be determined.

IP Dose-Response Tests

Subsequent to all animals reaching a discriminative criterion of eight correct lever selections in 10 consecutive sessions, the discriminative training regimen was limited to every other day to maintain discrimination. On intervening days, rats were tested with other IP doses of cathinone with each dose tested twice. In test sessions with doses different from the 800-μg/kg cathinone training dose, rats were immediately removed from the experimental space upon making 10 responses on either lever. If at any time during this dose-response testing the rat's maintenance discrimination fell below the 80% criterion, data on that animal was to be dropped from the results. This, however, did not occur.

Surgery and ICV Injections

After the dose-response relationship was determined with IP administration of various doses of cathinone, rats were anesthetized using 100 mg/kg ketamine HCl with a 0.15-ml injection of xylazine (10 mg/ml, Sigma Chemical Co., St. Louis, MO). Once anesthetized, each rat underwent a triple brain cannula implant. A single stainless steel outer gauge cannula (22 ga; Plastics One, Roanoke, VA) was stereotaxically targeted toward the right lateral ventricle using the coordinates: 0.5 mm posterior to bregma, 1.5 mm lateral to midline, and 3.2 mm ventral to the surface of the dura, with the skull kept level between lambda and bregma (18). Once the cranio-cement had set for this ICV implant, a bilateral placement of additional 22-ga cannulae were targeted to each of the two nucleus accumbens using the coordinates: 1.7 mm anterior from bregma, 1.5 mm lateral from midline, and 6.8 mm ventral to the surface of the dura (18). All rats were given at least 7 days postsurgical recovery time before the onset of testing.

ICV injections were performed using a modified method [after Myers (16)] in which drug solution was backloaded up a 28-ga internal cannula via a length of PE-20 tubing attached to a 25-μl Hamilton microsyringe. Solutions were administered in a total injection volume of 5 μl over a 20-s duration. In contrast, the bilateral nucleus accumbens injections were delivered by a 25-μl Hamilton microsyringe mounted in a microinjection pump (CMA/100, Bioanalytical Systems Inc., W. Lafayette, IN). Programming allowed for these nucleus accumbens injections to be maintained at 0.5 μl unilaterally over a 2-min period. Both the ICV and nucleus accumbens internal cannulae extended 0.5 mm beyond the guide cannula and all drug injections were performed while gently holding the rat by hand. The internal cannula remained in place for at least 30 s to allow complete drug delivery and pressure equalization; each injection was followed by visual inspection of the internal cannula for positive fluid flow. The rat was placed into the two-lever operant chamber exactly 5 min after ICV injection as this postadministration interval had been shown to elicit maximum discriminative performance after cocaine (28).

Testing of Cannula Patency and Histological Verification of Placement

Cannula patency was tested by measuring water intake following ICV administration of angiotensin II (a potent dipsogin) at 40 mg/5 μl before and after ICV cathinone discrimination. Rats that failed to drink at least 5 ml water within 15 min after AGII administration were excluded from subsequent testing. After intraaccumbens dose-response relationships were determined, all subjects underwent histological verification of cannula placement. Each subject was overdosed with sodium pentobarbital (200 mg/kg) and injected ICV with 4 μl Staedtler (#C745) ink. Approximately 10 min after injection of the ink, each subject was profused transcardially with physiological (0.9%) saline followed by a solution of buffered formalin (10%). Brains were rapidly removed and bathed in formalin. Subsequently, coronal sections (40 μm) were made in the brain along each cannula tract. Positive cannula placement was verified visually by the presence of ink throughout the ventricles and in the nucleus accumbens. Only those subjects (n = 14) for which positive placement was visually verified were included in the results.

Dose Selection of Cathinone for ICV and Nucleus Accumbens Administration

ICV administration was initiated, on a test day, by first injecting the volume of saline to be used to dissolve the various cathinone ICV doses (5 μl). The initial ICV dose of cathinone

TABLE 1

DOSE RESPONSE RELATIONSHIP OF CATHINONE DISCRIMINATION AFTER (A) IP, (B) ICV, AND (C) INTRAACCUMBENS ADMINISTRATION IN RATS (n = 14) TRAINED TO DISCRIMINATE 800 μg/kg IP CATHINONE FROM ITS VEHICLE (veh)

A. IP

Cathinone Dose (μg/kg)	Quantal	Quantitative (SD)
800	89.7	82.2 (8.1)
400	69.2	65.9 (16.8)
200	19.2	31.5 (12.4)
100	3.9	11.1 (2.6)
0.0 (veh)	2.6	3.2 (4.6)
ED_{50}	330 μg/kg	
(95% CL)	(250-420)	

B. ICV

Cathinone Dose (μg/kg)	Quantal	Quantitative (SD)
256	82.1	69.3 (1.8)*
128	67.9	62.3 (9.3)
64	32.1	39.7 (5.2)
32	17.9	35.0 (10.6)
0 (veh)	7.1	22.3 (10.0)
ED_{50}	90.51 μg	
(95% CL)	(68.43-119.72)	

C. Intraaccumbens

Cathinone Dose (μg/rat)	n/N†	Quantal	Quantitative
128	10/13	80.0	65.4
64	10/13	50.0	50.0
32	10/13	60.0	53.9
0 (veh)	13/13	0.0	15.6

*Not significantly different from quantitative measurement after 800 μg/kg administered intraperitoneally; Student's t-test, t = 2.133.

†n/N: number of rats responding/number of rats tested.

selected was 32 μg, with the rat returned to its home cage for 5 min prior to placement into the experimental chamber. Doses of cathinone ICV were increased to 64, 128, and 256 μg with each dose employed on two occasions: once following an IP cathinone maintenance session and once following an IP vehicle maintenance session.

At the conclusion of the dose-response experiments using the ICV route of administration, saline was administered bilaterally into the nucleus accumbens of each animal. Five minutes after administration, they were tested in extinction. Subsequently, doses of 32, 64, and 128 μg total (half given via each nucleus accumbens cannula) were tested with rats receiving a maximum of four nucleus accumbens injections. Following the nucleus accumbens dose-response relationship experiments, animals were sacrificed and histological placement was verified.

RESULTS

The results of dose-response experiments after intraperitoneal administration of three lower doses of cathinone than the 800-μg/kg training dose appear in Table 1A. Administration of the cathinone training dose produced 89.7% of selections upon the cathinone-appropriate lever, whereas trials with vehicle produced 2.6% of selected lever choices on this lever. Decreasing doses of cathinone produced decreasing discrimination in terms of both quantal and quantitative measurements. Analysis, by a computerized version (26) of the Litchfield-Wilcoxon method (14), indicated an ED_{50} value of 330 μg/kg for intraperitoneally administered cathinone. Table 1B represents the results of ICV administration with the cathinone doses expressed as μg per rat of cathinone administered. Saline administered ICV produced 7.1% cathinone lever selections and increasing doses of cathinone from 32-256 μg ICV produced increasing cathinone-appropriate responding. The highest ICV dose (256 μg/rat) produced 82.1% of cathinone-appropriate quantal selections with a quantitative measurement (69.3%) that was not significantly different from the quantitative measurement after the 800-μg/kg IP training dose. Generalization was, therefore, seen to occur as 80% or greater quantal selection on the cathinone-appropriate lever, a percentage generally used to assure transfer of effect since this is the criterion (i.e., 8 of 10 correct selections) used to adjudge animals capable of discriminating peripherally administered cathinone from its vehicle (see the Method section). Analysis of the dose-response curve after ICV administration indicated an ED_{50} value of 90.51 μg/rat and analysis (26) of potency ratio (PR) between IP and ICV indicated that they were significantly different (PR = 3.61, $p < 0.05$). Table 1C indicates the discriminative performance after administration of vehicle (all selections made upon vehicle-appropriate lever) or one of three doses of cathinone into the nucleus accumbens. The highest dose (128 μg, 64 μg bilaterally) produced a generalization effect in the 10 (of 13 tested) rats that selected a lever.

Figure 1 illustrates the dose-response relationship of IP- and ICV-administered cathinone calculated as μg per rat. In the case of IP administration, this was the absolute dose of cathinone administered as calculated in light of the mean weights (± SD) of rats = 478.1 (28.8) g with a range of 412-

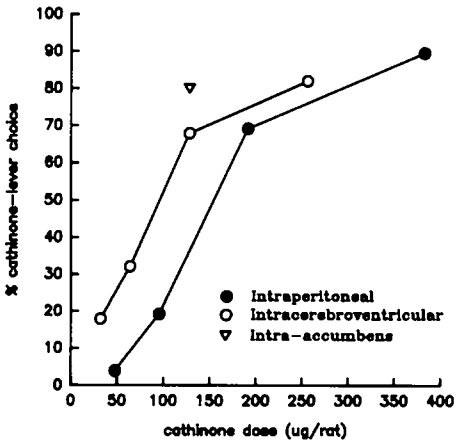

FIG. 1. Dose-response relationship of cathinone administered either IP, ICV, or into the nucleus accumbens of rats (n = 14) trained to discriminate IP-administered 800 μg/kg cathinone. Abscissa: cathinone dose calculated as absolute value per rat. In the case of IP administration, dose was calculated as per average weight. Ordinate: percent of first lever selections (pressed 10 times) upon the cathinone-designated lever.

519 g. For ICV, the values are μg/rat and the result of administering the highest dose of 128 μg intraaccumbens is shown for reference. When the IP dose-dependent quantal values are compared to the ICV values in a test for parallelism (26), the lines are parallel (calculated $t = 0.242 <$ critical $t = 2.776$).

DISCUSSION

The subjective effects of Khat chewing, which include euphoria, improved intellectual efficiency, and alertness, are reminiscent of the effects of both amphetamine and cocaine. In addition, the psychoactive ingredient in this shrub, viz., cathinone, has been shown in numerous laboratories to possess pharmacological properties that are analogous to these other psychostimulants (11,12). Indeed, the commonality of effects between these drugs in producing interoceptive cues, to which rats can make differential responses in a drug discrimination paradigm, has been shown (21). Since cocaine, which had been directly administered into discrete dopamine-rich brain areas, was determined to substitute in IP cocaine-trained rats (28) and amphetamine administered directly into the nucleus accumbens was shown to generalize in IP amphetamine-trained rats (17), it was of interest to see if either ICV- or intraaccumbens-administered cathinone would generalize in animals trained to IP-administered cathinone. The results indicate that, indeed, this generalization between peripheral training and central (both ICV and intraaccumbens) administration occurred.

To equate the potency between IP and ICV or intraaccumbens administration of cathinone, the cathinone training dose of 800 μg/kg was calculated as an absolute dose by determining that the mean weight of animals at the time of testing was 478.1 g and, thus, the 800 μg/kg would amount to 382.5 μg, which in turn produced 89.7% of cathinone-appropriate lever selections (Table 1A). A dose of 256 μg administered ICV was shown to generalize when animals chose the cathinone-appropriate lever on 82.1% of the trials and a dose of 128 μg/rat was shown to generalize after intraaccumbens administration. Thus, with this manipulation in place it appears that to produce generalization after ICV administration requires approximately 67% of the absolute peripheral dosage and for generalization after intraaccumbens administration approximately 34% of the peripheral dose is required. Thus, unlike the results with cocaine, in which the ICV dose was approximately 50 times more potent than the IP dose (29), or with amphetamine, where the intraaccumbens was 10 times more potent that the peripheral dose (17), the present indication is that cathinone is less potent than these two other drugs after microinjection into the rat brain. These results are more in accord with those of Rosecrans and Chance (19), where the calculation of ED_{50}s on a per kg basis revealed that the equivalent intraventricular dose of nicotine was approximately equal to the peripherally administered dose. The reason for the low potency of cathinone by the ICV route is not, at present, known.

The observation of generalization from IP- to ICV-administered cathinone is of more importance when one considers that the dose-response curves are parallel as the suggestion has been made that when this occurs drugs may be acting on the same receptors (13). This is of interest since ICV-administered cathinone is limited only by local diffusion in the ventricles for its interaction with receptors, whereas after IP administration the amount of drug able to affect the central (presumably dopamine) receptors is dependent upon pharmacokinetic factors, such as absorption, distribution, and metabolism of cathinone by peripheral mechanisms.

Although the number of cathinone intraaccumbens administrations must be limited in number by the possibility of tissue damage at this site, the observation that 128 μg/rat (64 μg in each accumbens) was able to produce a cueing effect that was similar to that observed after IP cathinone administration would suggest that the dopaminergic cells in the accumbens are important for the discrimination of this psychostimulant. However, as stated by Wise (27): "the only way to identify the site of action of centrally injected drugs is to compare the potency and latency of the effects of the injection in a variety of overlapping or closely adjacent sites." Thus, administration of cathinone via indwelling cannulas placed at other dopaminergically rich sites, such as the medial prefrontal cortex, anteromedial caudate nucleus, amygdala, and/or the area postrema, would allow the present evidence, regarding the importance of the nucleus accumbens, to be highlighted. These studies are currently underway in this laboratory.

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