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Cocaethylene Produces Discriminative Stimulus Properties in the Rat: Effect of Cocaine and Ethanol Coadministration

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SCHECHTER, M. D. *Cocaethylene produces discriminative stimulus properties in the rat: Effect of cocaine and ethanol coadministration.* PHARMACOL BIOCHEM BEHAV 51(2/3) 285–289, 1995.—Experimentally naive Sprague-Dawley male rats were trained to discriminate the interoceptive stimulus cues produced by either 10.0 mg/kg cocaine or 10.0 mg/kg cocaethylene from their saline vehicles. Although it required more sessions to train the cocaethylene rats, once they were trained to criterion performance the ED_{50} value for cocaethylene (2.89 mg/kg) was very similar to that of cocaine (3.04 mg/kg). Coadministration of a 300-mg/kg dose of ethanol that produced saline-like responding in cocaethylene-trained rats with 2.5 mg/kg cocaine allowed for 88.9% of first lever selections being made on the cocaethylene-appropriate lever. Time-course evidence using coadministered (1.25-mg/kg) cocaine and (300-mg/kg) ethanol indicated that the formation of cocaethylene was highest, as indicated by discriminative performance, at 15 min and progressively decreased as the postinjection interval was increased to 30, 60, and 120 min. The results are discussed in light of rapid formation of cocaethylene from cotreatment with ethanol and cocaine in the mouse, rat, and human subject. The suggestion is made as to the prevalent, and growing, use of this drug combination in the human population of cocaine abusers.

Cocaethylene Cocaine Ethanol Stimulus properties of drugs Time-course Rats

WHEN an alcoholic beverage (ethanol) is ingested at the same time that cocaine is administered, the human liver carboxylesterase enzymes produce a third substance; this represents the only known example of the body producing a novel psychoactive drug exclusively during the administration of two drugs of abuse. The ethanol-derived cocaine metabolite is produced only when ethanol is consumed, in that it is not a natural alkaloid of coca, nor is it found in the normal catabolism of cocaine. The substance is known by numerous names in the scientific literature, including: ethylbenzoylecgonine (5), benzoylecgonine ethyl ester (6), cocaine ethyl-ester (7), ethyl cocaine (15), ecgonine ethyl ester benzoate (11), ethylcocaine (2), and the name used here, cocaethylene (3). Cocaethylene was identified as early as 1979 in the urine of patients found to test positive for both ethanol and cocaine (12), and it has been identified in a group of postmortem blood samples in concentrations that exceed those found for cocaine (6). Recent studies indicate that cocaethylene possesses at least some of the pharmacologic effects of its parent compound, cocaine. It is equipotent in its dopamine transporter site affinity in both rat (5) and human (6) brain tissue, and in inhibiting dopamine reuptake into rat striatal synaptosomes (10).

Behavioral studies have also indicated similarities in the effects of cocaine and cocaethylene in that both compounds produce increased locomotor activity in the rat and function as reinforcers in self-administration experiments in rhesus monkeys (6). At least three laboratories (7,13,18) have been sites for experiments in which rats were trained to discriminate the interoceptive stimulus cues produced by cocaine and were shown to subsequently generalize their discriminative performance when administered cocaethylene. Thus, the interoceptive cues produced by cocaine appear to be similar to those produced by cocaethylene. However, at this time there are no reports of the ability of cocaethylene to function as the drug to control differential responding in a drug discrimination task. The present study intended to train rats to discriminate a dose of cocaethylene equivalent to that previously used to train cocaine (13) and to test dose–response relationships, as well as the time course of cocaethylene-controlled discriminative behavior after coadministration of ethanol and cocaine.

METHOD

Subjects

Male experimentally naive Sprague–Dawley rats, weighing 175–190 g at the beginning of the experiment, were purchased

from Zivic-Miller Laboratories (Allison Park, PA). Ten rats were assigned to be trained to discriminate 10.0 mg/kg cocaethylene fumarate (as salt; NIDA), whereas 12 were assigned to a second group to be trained with a similar amount of cocaine to compare learning rates in rats during the same period of time. The individually housed rats were kept in a Vivarium facility with an ambient temperature of 20–22°C and maintained on a 12 L : 12 D cycle with lights on at 0600 h. Tapwater was continually available in their home cage but commercial rat chow was rationed so as to allow maintenance of their body weights at 85–90% of free-feeding weights as determined by a growth chart provided by the supplier. This procedure endeavored to facilitate motivation of operant performance for food reward.

Apparatus

Twelve standard rodent operant chambers (Lafayette Instruments Corp., Lafayette, IN), each containing two levers situated 7 cm apart and 7 cm above a metal grid floor, were used as the experimental space; these were located in a room separate from the Vivarium facility. Equidistant between the levers was mounted a food receptacle that received delivery of 45-mg Noyes food pellets (Lancaster, NH), and each operant chamber was enclosed in a sound-attenuated cubicle with an exhaust fan and a 9-W house light. Solid-state programming equipment (Med Associates, St. Albans, VT) was located in an adjacent room and was used to control and record discriminative training/testing sessions.

Discrimination Training

The food-deprived rats were trained to press one of two levers under the drugged condition—i.e., cocaine or cocaethylene—as well as a second identical lever in the nondrugged (saline vehicle) state. Training sessions were conducted once a day, 5 days/week, with one lever in each cage designated the "saline lever" and the second lever designated the "drug correct lever." Thus, ten rats were trained to discriminate between 10.0 mg/kg cocaethylene and its saline vehicle, whereas the other 12 rats were trained to discriminate 10.0 mg/kg cocaine from the same vehicle. Both drugs were administered intraperitoneally (IP) 15 min before the training session. Initially, all rats were trained to respond on the saline lever after the IP administration of 1 ml/kg of the vehicle on a fixed ratio (FR) of 1 (i.e., one response resulted in the delivery of one 45-mg Noyes food pellet as reinforcement). During eight consecutive training sessions, the FR schedule was gradually incremented to attain an FR 10 schedule of reinforcement in that ten responses on the saline lever produced one reinforcement. The rats were removed from the operant chamber and returned to their home cages after making 400 responses, thus receiving 40 reinforcements on this FR 10 schedule.

Once an FR 10 schedule was established on the saline lever, training began on the opposite lever 15 min following the IP administration of the vehicle containing either 10.0 mg/ml cocaine (n = 12) or 10.0 mg/ml cocaethylene (n = 10), and the rats were only rewarded for responding upon the drug-appropriate lever. The initial reinforcement schedule started at an FR 1 and was gradually increased over seven consecutive daily training sessions to an FR 10 schedule of reinforcement. Once FR 10 lever-press performance was established on both levers, discrimination training began in which daily injections of either saline (S) or drug (D) was administered on a 2-week, repeating, administration schedule: D,S,S,D,D; S,D,D,S,S. The first lever upon which 10 responses were accumulated at the beginning of each of these sessions was considered the "selected lever" for that daily session. At the time of the 10th response, presses on both selected and unselected levers were recorded but incorrect responses produced no programmed consequence. The session was continued regardless of the correctness of the selected lever until 400 responses were made on the correct lever for that day's session, and therefore, until 40 reinforcements (on the FR 10 schedule) were received. The training criterion (i.e., the discriminative performance that had to be attained to judge the animal capable of discriminating between the drug state and the nondrug state) was a minimum of eight correct lever selections appropriate for the substance injected on that day during ten consecutive training sessions.

Dose-Response Tests

After all rats reached the discrimination criterion, the discriminative training regimen was limited to every other day. The rats were administered the dose employed in their training or its vehicle and allowed to choose freely between levers; they were reinforced only for presses on the lever appropriate for the substance administered 15 min before their daily session. On intervening days, rats were tested with doses of cocaine in the cocaine-trained animals, or cocaethylene in the cocaethylene-trained animals, different from the 10.0-mg/kg training dose. The cocaine-trained animals received 5.0 and 2.5 mg/kg cocaine, whereas the cocaethylene-trained animals received 15.0, 5.0, and 2.5 mg/kg cocaethylene IP 15 min before testing. Each novel dose of drug was administered twice: once following a maintenance session with the dose used in training and once following a saline vehicle maintenance session. This counterbalancing procedure was employed to control for any possible residual influence from the previous day's maintenance session. If at any time during the maintenance test days a rat's discrimination fell below the 80% criterion (i.e., fewer than eight correct state-appropriate lever selections in 10 consecutive maintenance sessions), the data on that rat were to be dropped from the results. This occurred in two of the cocaine-trained rats and was reflected as n = 10 during dose-response testing.

Testing of Coadministrated Ethanol and Cocaine in Cocaethylene-Trained Rats

After the dose-response experiments were completed, test days in the cocaethylene-trained rats were used to test the effects of two treatments, ethanol and cocaine, at the 15-min postinjection time used in training. In addition, later times were employed to investigate the discriminative performance of the rats as a consequence of the formation of cocaethylene from coadministration of ethanol and cocaine. During the conduct of these experiments, one of the cocaethylene-trained rats died from unrelated causes, and the results reflected n = 9. Prior to cotreatment with ethanol and cocaine, it was thought necessary to determine the effects of two injections given at the same time on contralateral lower quadrants of the rat's anatomy. Thus, saline was administered immediately before two doses of cocaine (1.25 and 2.5 mg/kg) and 15 min later, the rats were tested on two occasions in the discrimination task. Likewise, a 300-mg/kg dose of ethanol was administered just before saline administration and the animals were similarly tested. Subsequently, ethanol at 300 mg/kg was administered with 1.25 and 2.5 mg/kg cocaine 15 min before testing. The 300-mg/kg ethanol dose plus the lower (1.25 mg/kg) cocaine dose were each administered on two occasions,

TABLE 1

SESSIONS-TO-CRITERION, DISCRIMINATIVE LEARNING RATES, AND DOSE-RESPONSE RELATIONSHIPS OF RATS TRAINED WITH EITHER 10.0 mg/kg COCAINE (n = 12 AND 10) OR 10.0 mg/kg COCAETHYLENE (n = 10)

STC $\bar{x}$ (SD) Range	Cocaine [5.6 (3.2) (2–12 Sessions)] Responses on Drug Lever				Cocaethylene (CE) [15.0 (5.1) (8–23 Sessions)] Responses on Drug Lever			
	After Saline		After Cocaine		After Saline		After CE	
	Quantal	Quantity (SD)	Quantal	Quantity (SD)	Quantal	Quantity (SD)	Quantal	Quantity (SD)
Week								
1,2	70.0	66.4 (23.0)	88.3	77.6 (8.9)	56.0	51.8 (19.6)	48.0	48.3 (14.9)
3,4	28.3	38.0 (10.7)	86.7	77.6 (5.4)	34.0	44.3 (13.4)	56.0	52.1 (5.3)
5,6	NA	NA	NA	NA	24.0	34.3 (2.6)	82.0	69.0 (8.1)
Dose cocaine	Quantal		Quantitative (SD)		Dose CE	Quantal	Quantitative (SD)	
10.0	95.0		86.8 (2.5)		15	75.0	70.0 (1.1)	
5.0	55.0		57.4 (14.6)		10	80.0	66.5 (7.2)	
2.5	50.0		49.5 (4.0)		5	70.0	60.9 (12.5)	
0	10.0		28.3 (6.9)		2.5	40.0	47.7 (12.5)	
					0	10.0	15.9 (15.6)	
	ED_{50}	3.04				2.89		

and the animals were placed into the discrimination testing apparatus at 30, 60, and 120 min post-cotreatments. Each of these postinjection times was conducted on two occasions: once following a 10.0-mg/kg cocaethylene maintenance (testing/training) session and once following a saline maintenance session. On all test days, the animals were removed immediately upon responding on one lever 10 times, whereas on intervening maintenance sessions they were allowed to lever-press on the state-appropriate lever to receive a total of 40 reinforcements.

Data Analysis

The data collected in the drug discrimination sessions are expressed as both quantal and quantitative measurements. Each of the individual measurements provides a different indicator of lever preference before reinforcement. The quantal measure is the percentage of rats that chose the drug-appropriate lever as their selected lever—that is, the lever first accumulating 10 presses. The quantitative measurement is the number of responses on the drug-lever divided by the total number of responses on both the drug- and the saline-lever at the time that 10 responses were accumulated on either lever; this fraction is expressed as a percentage. On all test days with different doses of the trained drug or with drug combinations, the rats were immediately removed upon pressing one of the levers 10 times. This precluded any possible reinforcement/training in a condition different from the training condition as reinforced in maintenance sessions. Unlike the all-or-none quantal measurement, the quantitative measurement allows for responses on both the selected and unselected levers to be considered; thus, it provides a relative measure of magnitude, as well as direction, of lever preference. In addition, statistics (i.e., Student's t-test) can be performed on the quantitative data. A computer-based (17) formulation of the Litchfield-Wilcoxon procedure (8), which employs probits vs. log-dose effects, was used to generate ED_{50} values and confidence limits from the cocaine quantal dose-response relationship in cocaine-trained rats and from the cocaethylene quantal dose-response relationship in the cocaethylene-trained rats.

RESULTS

As indicated, the rats constituting the cocaine-trained group were included in this study to indicate learning rates and dose-response relationship of the parent compound to be compared with cocaethylene training during the same period of time. Table 1 indicates that the first session in which the animals accumulated 10 presses upon the state-appropriate lever in eight of 10 consecutive sessions; this sessions-to-criterion occurred in a mean of 5.6 sessions, with a range of two to 12 sessions for cocaine discrimination. In contrast, it took approximately three times as many sessions—i.e., 15 sessions on the average—for cocaethylene at the same dose to allow the rats to attain this criterion discriminative performance. This is further indicated in Table 1 by the actual quantal and quantitative measures—i.e., responses on the drug lever after saline and drug administration, as it represents five trials of each over a 2-week training period. As cocaine criterion was met, at the latest by the 22nd session, training in weeks 5 and 6 was not needed. Nevertheless, by the 3rd and 4th week, the 12 rats (at that time) chose the drug lever in 86.7% of the sessions after cocaine and the same lever in 28.3% of the sessions after saline (weeks 3 and 4). In cocaethylene-trained animals, it was necessary to continue training through the 5th and 6th weeks, and for one animal (the one rat that required 33 sessions), 3 additional days. In any case, it appeared that 10.0 mg/kg cocaine was more discriminable than a similar dose of cocaethylene. Once trained, the dose-effect relationship of both drugs indicated that decreasing doses generally produced decreased discriminative performance and similar ED_{50} values with cocaine = 3.04 mg/kg and cocaethylene = 2.89 mg/kg (Table 1, bottom).

Table 2 shows the effects of cotreatments with two conditions in the nine viable cocaethylene-trained rats. Administration of saline before 1.25 and 2.5 mg/kg cocaine produced 11.1 and 61.1% of selected lever choices on the cocaethylene-correct lever, respectively, whereas 300 mg/kg ethanol produced saline-like (5.6% responses on cocaethylene lever) responding. When 300 mg/kg ethanol was administered with 2.5 mg/kg cocaine, and animals were tested 15 min later,

TABLE 2

DISCRIMINATIVE PERFORMANCE AFTER COTREATMENTS AT DIFFERENT POSTINJECTION (P-I) TIMES IN RATS (n = 9) TRAINED TO DISCRIMINATE 10.0 mg/kg COCAETHYLENE FROM ITS SALINE VEHICLE

P-I Time (min)	Treatment 1	Dose	Treatment 2	Dose	Quantal	Quantitative (SD)
15	Saline	–	Cocaine	2.5	61.1	55.1 (8.4)
15	Saline	–	Cocaine	1.25	11.1	35.0 (8.3)
15	Ethanol	300	Saline	–	5.6	23.8 (9.1)
15	Ethanol	300	Cocaine	2.5	88.9	74.7 (13.2)
15	Ethanol	300	Cocaine	1.25	72.2	63.5 (12.6)
30					38.9	45.2 (4.2)
60					38.9	37.4 (3.1)
120					22.2	35.4 (6.6)
Maintenance sessions						
15	–		Cocaethylene	10.0	85.6	74.3 (5.8)
15	–		Saline	–	2.2	12.6 (8.0)

88.9% of quantal responding was made on the cocaethylene-appropriate lever. In most drug discrimination literature, ≥80% is considered the level of discriminative generalization if produced by a novel drug. In this case, where 80% was the criterion for judging the animal to be capable of discriminating cocaethylene (8/10 criterion), this combination of ethanol and cocaine would correctly be described as generalized. The same dose of ethanol with a lower dose of 1.25 mg/kg cocaine allowed for 72.2% of cocaethylene-appropriate responding, and when the postinjection interval was extended to 30, 60, and 120 min, this cotreatment produced lower cocaethylene-appropriate discriminative performance.

DISCUSSION

The results of the present investigation would seem to indicate that cocaethylene is capable of functioning as the drug to control differential responding in a drug discrimination procedure. When compared to the same training dose of cocaine, however, it appears that cocaethylene is less discriminable. Once animals are trained to cocaethylene, the ED_{50} values of cocaethylene and cocaine are similar. Previous work in which rats were trained to discriminate cocaine and then tested with various doses of cocaethylene indicated that cocaine was more potent than cocaethylene in those animals (7,13,18). In contrast to the behavioral effects, cocaine and cocaethylene have been shown to be equipotent in producing convulsions, and cocaethylene is actually more lethal in rats (7) and mice (14).

Results also indicate the additive effects of 1.25 mg/kg cocaine when coadministered with 300 mg/kg ethanol in cocaethylene-trained animals—i.e., the former produced 5.6% and the latter drug 11.1% of cocaethylene lever selections when administered alone vs. 72.2% when coadministered. When 2.5 mg/kg cocaine plus 300 mg/kg ethanol were coadministered, the quantitative measure (74.7 ± 13.2) was not significantly different (t = 0.067, Student's t-test) (16) when compared to the quantitative measurement during the numerous 10.0-mg/kg cocaethylene maintenance sessions (i.e., 74.3 ± 5.8). When 300 mg/kg ethanol was administered with 1.25 mg/kg cocaine and the injection-to-testing interval was increased from 15 to 30, 60, and 120 min, cocaethylene-appropriate discriminative performance decreased. This would suggest that the formation of cocaethylene from cocaine and ethanol that allows for cocaethylene-appropriate discriminative performance is greater at 15 min and progressively subsides over the course of 2 h. This rapid formation of cocaethylene has, in fact, been reported in various species. Boyer and Petersen (1) indicated that peak hepatic concentrations of cocaethylene are reached in 2.5 min after cotreatment in mice, and Masur et al. (9) reported additive stimulatory effects of combined administration in this species. In addition, Dean et al. (3) reported substantial concentrations of cocaethylene in rat liver, brain, and serum 15 min after cotreatment. Most recently, McCance-Katz et al. (10) examined the pharmacokinetics of cocaine and ethanol administration in six human volunteers, and found cocaethylene initially detectable in 30 min with peak plasma concentrations at 115 min.

The rapid formation of cocaethylene from cotreatment with low doses of ethanol and cocaine allowed for discrimination of the combination as cocaethylene as it is formed in tests in cocaethylene-trained rats. This effect appears to occur very soon after co-use and may help to explain the prevalent (4) and growing (16) simultaneous use of alcohol and cocaine among cocaine users in the United States. Cocaethylene formation may prolong the euphorigenic effects of cocaine, while at the same time decreasing the dysphoric experience after cocaine intoxication (2).

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