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BRIEF COMMUNICATION

Cocaethylene Produces Conditioned Place Preference in Rats

MARTIN D. SCHECHTER

Department of Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272-0095

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SCHECHTER, M. D. *Cocaethylene produces conditioned place preference in rats.* PHARMACOL BIOCHEM BEHAV 51(2/3) 549–552, 1995.—The ability of cocaethylene to produce either a conditioned place preference or a conditioned place aversion was tested in rats. Twelve male rats were administered 10 mg/kg cocaethylene and confined to their nonpreferred side of the conditioned place preference apparatus as determined on a baseline test day. Subsequently, these rats spent a greater amount of time in that cocaethylene-paired nonpreferred side when later tested in a drug-free state. In contrast, rats conditioned with the same dose of cocaethylene and confined in their preferred side, as well as other rats treated with saline on both sides, did not show a significant shift in their preference or aversion. Results are discussed in light of the rewarding activity of cocaethylene, a compound formed in humans who concurrently use cocaine and ethanol.

Cocaethylene Cocaine Conditioned place preference Ethanol Rats

A RECENT NATIONAL survey has indicated that approximately 12 million Americans concurrently use cocaine and alcohol, and it appears to be the most prevalent pattern found among individuals admitted to emergency rooms with substance abuse (3). This widespread (reported to be as high as 77%) (3), concurrent, and simultaneous use of two abused drugs highlights the fact that although much research has been done regarding the effects of each of these two drugs when given alone, surprisingly little research has been done in humans when both drugs are given concurrently. In light of the large number of reports from emergency rooms regarding the adverse cardiovascular effects of the combination (6,8), the coadministration of these two drugs is of current scientific and medical interest. The first metabolite of cocaine benzoylecgonine can be ethylated by acetaldehyde, as it is derived from ethanol metabolism in the liver, in minutes to produce the cocaine metabolite cocaethylene (1,2). This chemical has been isolated from the brains of five of seven cases of cocaine-related sudden death arriving at the pathologist's office in Dade County, Florida (10), and it has been found in the blood of human volunteers in a double-blind placebo-controlled study (7). The possibility has been suggested that this unique metabolite has the capability of intensifying cocaine's euphoric effect, worsening craving for it, and possibly causing addiction that is harder to break, while at the same time lessening the dysphoric sequelae of agitation and even, perhaps, the occurrence of paranoia (7,8). In animal experimentation, cocaethylene (like cocaine) has been reported to bind to the dopamine transporter that allows presynaptic reuptake and inactivation, thereby increasing the extracellular concentration of dopamine in the nucleus accumbens. This neurochemical activity has been suggested to explain its ability to increase locomotor activity and sustain its self-administration (5).

The conditioned place preference (CPP) test has found widespread use in measuring the rewarding activity of a large number of drugs, as indicated by a recent bibliography citing 330 articles (13); cocaine is the drug used in 31 of these studies and ethanol has been employed in 28 other studies. However, in no case has cocaethylene been employed in this behavioral task. The CPP test allows for the conditioning of a distinct environment with a drug; implicit in this paradigm is the assumption that the positive rewarding properties of the drug become associated with distinct environmental cues that have been paired with the drug treatment during conditioning trials. If pairing a drug with an environment decreases the time the subject spends in that environment, the drug is said to be aversive and to produce a conditioned place aversion. The purpose of the present study was to use a dose of cocaethylene

that had previously been reported to increase locomotor activity (5) and allow for differentiated discriminative behavior (11) in rats, and to pair that dose with both the preferred and, in a different group of animals, nonpreferred side of a CPP test apparatus to indicate the preference or aversive nature of this novel cocaine-ethanol metabolite.

METHOD

Subjects

Thirty-six male rats of Sprague-Dawley descent were individually housed in stainless-steel hanging cages after being purchased, at a weight range of 225–275 g, from Zivic-Miller Laboratories (Allison Park, PA). All animals were allowed free access to food and water throughout the experimentation and were kept in a room with an ambient temperature of 20–22°C and maintained on a 12 L : 12 D cycle with lights on at 0600 h.

Conditioned Place Preference Apparatus and Procedures

Place conditioning and testing were conducted in one of four modular test-component units (Model 85000; Lafayette Instrument Co., Lafayette, IN). The three-chambered stainless-steel apparatus consisted of a center section from which the subjects were allowed free access into two end sections. Restraining metal plates (Model 85009) served to restrict a rat's egress from either the right or left side of the apparatus during conditioning sessions. The right and left end sections (20.5 × 30.5 × 20 cm), originally identical, were altered in three sensory modalities (i.e., tactile, visual, and olfactory) so as to allow discriminable cues. The "dark" side was illuminated by a 6-W, 30-V red lightbulb and had a smooth, black Plexiglas floor. The "light" side was illuminated by a 6-W, 30-V white light and had a stainless-steel grid floor under which pine wood shavings were placed. Location throughout the chamber was detected by weight-pivot sensors connected to a computer that automatically recorded the time (in seconds) spent in each section of the apparatus.

Twenty-four subjects were randomly assigned to one of two groups, with the first being conditioned with 10 mg/kg cocaethylene (as the fumarate salt, NIDA) on the side that they preferred (the P side), whereas the other half of the animals received the same dose on the side that they did not prefer (the NP side). In addition, the 12 remaining animals were divided into two groups ($n = 6$), with each receiving saline administration on both their P and NP sides. Half of these animals were assigned the dark-side chamber as preferred, whereas the other six were assigned the lighted chamber as preferred. Each subject underwent three treatment phases. Initially, all rats were given 3 days of habituation to the entire CPP apparatus and, on the last of these days, 15 min of free access served to establish a preconditioning baseline of place preference for each individual rat. The side on which the rat spent more time (in seconds) was considered its P side for the remainder of the study, whereas the side in which the animal spent less time was considered its NP side. With half the animals assigned to be conditioned with drug on their P side and the other half of the rats assigned to be conditioned on their NP side, the second phase, drug conditioning, was initiated and conducted daily for 30 min over a 6-day period. On alternate days, animals were confined to either their P or NP side for 30 min after intraperitoneal (IP) administration of 10 mg/kg cocaethylene. On alternate days, the animals were administered an equal volume (1 ml/kg) of saline and confined to their opposite side for the same duration of time. The saline-saline control group was given the same volume of saline IP and confined, alternatively, to their P and NP sides. Twenty-four hours following the last day of conditioning, each animal was allowed free access, as on the baseline day, for 15 min to determine place preference in a nondrugged state.

Measurements and Statistics

The critical measure was the time spent in both the P and NP sides during the baseline and (last day) preference test. The measurement used was the difference score between the time spent in the NP side and the amount of time spent on the P side. These difference scores allow for consideration of data on the amount of time spent on the side originally preferred or nonpreferred during the baseline test and, therefore, the side paired during interspersed conditioning sessions with either cocaethylene or saline. Because the CPP test employed a single cocaethylene dose, Student's paired t-test was conducted to compare difference scores between the baseline and preference test day. Likewise, the saline-saline control data were scored in the same way, and analysis was conducted with a paired t-test.

RESULTS

The mean (± SEM) number of seconds spent on the P side during baseline testing by animals conditioned with cocaethylene on that side was 399.7 (21.7) s, whereas the number of s spent during the baseline session on the NP side in animals conditioned with cocaethylene on that side was 279.8 (15.3) s. When the mean number of seconds spent on the P side was subtracted from the number of seconds spent on the NP side in the first group (Table 1), the number was negative (by definition) and equal to −119.9. In the animals conditioned with cocaethylene on their NP side, the mean difference score (NP − P) was −188.7 s. These scores for baseline were not significantly different ($t = 1.156$). When 10 mg/kg cocaethylene was paired with the P side in 12 rats, the mean (± SEM) difference score increased to −94.6 (49.9), a 21.1% increase from baseline but not significantly different ($t = 0.606$). In contrast, when rats were conditioned with this dose of cocaethylene and confined to their NP side, the baseline difference score measurement increased from −188.7 to −77.2 during the postconditioning preference test, a change of 59.2% and a significant ($t = 2.322$, $p < 0.05$) effect. The six saline-saline control rats who were randomly assigned to have the saline administration and confinement in their P side (when, in reality, saline was given before placement on both sides), as well as the six other saline-saline control rats who were designated as saline confinement on their nonpreferred side (as again, it was given on both sides), showed a nonsignificant change of 32.7 and 29.9%, respectively, in their difference scores (Table 1).

DISCUSSION

The CPP test is particularly well suited for studying the neuropharmacology of drug reward, and there is much evidence that it is capable of measuring the reinforcing actions of psychostimulant medications (13). Thus, the stimulants cocaine and amphetamine have been especially well evidenced to produce a CPP (4). The present results indicate that the metabolic product of cocaine and ethanol is also capable of

TABLE 1

DIFFERENCE BETWEEN MEAN (± SEM) TIME (SECONDS) SPENT IN NONPREFERRED SIDE MINUS TIME SPENT IN PREFERRED SIDE IN RATS CONDITIONED WITH COCAETHYLENE (10 mg/kg) OR SALINE ON THEIR PREFERRED OR NONPREFERRED SIDE

Rats Conditioned on their Preferred Side			Rats Conditioned on their Nonpreferred Side		
	Baseline	Post conditioning		Baseline	Post conditioning
CE (n = 12) $\bar{x}$ (SEM)	−119.9 (34.8)	−94.6 (49.9)	CE (n = 12) $\bar{x}$ (SEM)	−188.7 (38.6)	−77.2 (45.1)*
	% change: 21.1%			% change: 59.2%	
SAL (n = 6) $\bar{x}$ (SEM)	−252.2 (133.1)	−169.7 (161.1)	SAL (n = 6) $\bar{x}$ (SEM)	−147.2 (43.5)	−103.2 (94.9)
	% change: 32.7%			% change: 29.9%	

*Significant difference from nonpreferred-perferred measurement on baseline trial, $p < 0.05$, Student's paired t-test. CE, Cocaethylene; SAL, saline.

producing a significant shift toward CPP in rats. This was seen in view of the fact that only rats conditioned with cocaethylene on their NP side shifted their preference to that side in relation to the P side (difference score measurement) when conditioning was done on the NP side. In contrast, when the same dose (10 mg/kg) of cocaethylene was administered to animals while on their P side, there was no significant change in their difference score. Likewise, when saline was used as a control and given to animals paired on both their P and NP sides, there was a similar percent change in the difference measurement, indicating that no significant change in preference occurred. In light of the fact that analysis of time spent in the center compartment did not vary in any of the four groups of animals (data not shown), it must be surmised that the increase in amount of time spent in the NP side, in all groups, came from that previously spent in the P side as determined during baseline testing. Furthermore, only in those animals having cocaethylene paired with their NP side was this shift statistically significant.

Cocaethylene has been found to be self-administered by primates and, in neurochemical studies, has been shown to have the same ability as cocaine to inhibit the reuptake of dopamine in vitro (5). Indeed, because cocaethylene is structurally similar to cocaine, it is not surprising that it has a similar spectrum of neurochemical and behavioral effects. Cocaine is as potent as cocaethylene in inhibiting the specific ligand binding to the dopamine reuptake transporter complex, in turn decreasing dopamine uptake into synaptosomes, and thereby increasing extracellular dopamine concentrations after its administration to rats (5). What is interesting, however, is the observation that the half-life of cocaethylene may be longer than that of cocaine. Thus, its production may prolong cocaine's effects as greater access may be made by it through the blood-brain barrier because of increased lipid solubility. If cocaethylene is capable of these activities and its cited (9) dopamine transporter affinity is the primary biochemical influence upon its reinforcing effect, then elevated levels of cocaethylene may be pharmacologically added to those of cocaine and thus result in more accumulation of dopamine in the synaptic cleft. This may explain the enhanced euphoria observed in anecdotal reports from cocaine abusers suggesting that in the course of cocaine binging, the addition of alcohol prolongs the euphoria and/or ameliorates dysphoric symptoms of acute abstinence (5). In this laboratory, cocaethylene's discriminative effects have been directly shown to outlast those of cocaine in a dose-response manner (11). In addition, rats trained to discriminate 10 mg/kg cocaethylene from its vehicle required more sessions to reach criterion performance than other rats trained to discriminate 10 mg/kg cocaine (12). This suggests that the potency of cocaine in the drug discrimination task is greater than that of cocaethylene. In fact, the present CPP produced with 10 mg/kg cocaethylene was less in magnitude than that reported to occur with lower doses of cocaine (4,13). Unlike these previous discrimination studies, only a single (10-mg/kg) dose of cocaethylene was used in the present CPP test and a dose-effect response was not determined. Nevertheless, this dose had previously been shown to cause increased activity in a 15-min period (5), as well as serving to control differential responding in a drug discrimination paradigm (12).

Ethanol has been reported to simultaneously suppress normally rapid enzymatic inactivation of cocaine (thus increasing cocaine blood-brain levels) and catalyze cocaine's ethyl tranesterification to the pharmacologically active cocaethylene shortly after coadministration in the rat. The present evidence that cocaethylene produces a rewarding effect may help to explain the large number of cocaine abusers who imbibe alcoholic beverages concurrently.

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