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Cocaethylene-Induced Kindling of Seizure Effects: Cross-Specificity With Cocaine

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MEEHAN, S. M. AND M. D. SCHECHTER. *Cocaethylene-induced kindling of seizure effects: Cross-specificity with cocaine.* PHARMACOL BIOCHEM BEHAV **54**(2) 491–494 1996.—Sensitization and cross-sensitization to the seizurogenic effects of cocaine and cocaethylene were examined in the HS strain of mice. Animals were administered IP injections of either 48 mg/kg cocaine or 32 mg/kg cocaethylene once per day for 4 days. On the fifth day, mice were injected with either the same drug that was administered on days 1–4 or the alternative psychostimulant and the occurrence of seizure activity was recorded. Repeated cocaine administration resulted in the induction of tonic-clonic seizures and status epilepticus in 90% of the animals tested with cocaine on the fifth day. A similar increase in seizure prevalence, noted as a kindling effect, was observed in cocaethylene-treated animals tested with cocaethylene in that 90% of the mice exhibited status epilepticus on the last test day. Significant cross-sensitization was observed only in the group that received cocaethylene following repeated cocaine exposure. However, data obtained from animals injected with cocaine following cocaethylene treatment also were suggestive of cross-sensitization effects. Results are discussed in terms of the potential mechanistic differences between cocaine and its ethanol-derived product, as well as its relevance to cocaine use/abuse.

Cocaethylene Cocaine Kindling Mice Seizure

PROGRESSIVELY increased behavioral excitability has been evidenced when a constant dose of cocaine is intermittently and repeatedly administered over time (3,19). This cocaine-induced sensitization has also been shown when subconvulsant doses have produced seizures following repeated administrations in rodents (6,13). This sensitization to the seizurogenic effects of cocaine has been termed cocaine kindling, based on its similarity to the behavioral effects observed following electrical kindling of the amygdala (4,12,14). Relatedly, there is a growing body of evidence suggesting that serotonergic mechanisms play a major role in the seizurogenic effects of cocaine (15,17) and dopaminergic systems mediate cocaine-induced behavioral sensitization (20).

Cocaethylene (CE) is a psychoactive metabolite of cocaine produced in the liver when cocaine is administered in conjunction with ethanol (2). CE has a pharmacological profile similar to that of cocaine in that it blocks presynaptic dopamine transport resulting in increased synaptic levels and enhanced postsynaptic stimulation. However, CE has been reported to be less potent in regards to its effect upon serotonergic neuronal systems (1) and more potent in producing lethality in rodents (5,7,16). Furthermore, in mice, CE produces a characteristic seizurogenic profile that is somewhat different than that produced by cocaine (11). Briefly, cocaine typically induces tonic-clonic seizure activity; motor seizures characterized by extension of one or both hind limbs at an angle greater than 90°, accompanied by gaping (mouth open with head raised 30–45° from horizontal). These tonic-clonic seizures are rapidly followed by status epilepticus, which has been characterized as violent motoric activity during which the animal rebounds off the bottom and sides of the cage (11). In contrast to the seizure activity produced by cocaine, CE has been shown to dose-dependently produce status epilepticus in the absence of any tonic-clonic activity (11).

Despite the reported similarities in pharmacological activity between cocaine and CE, there have been no systematic investigations as to cocaethylene-induced kindling. Therefore, the aim of the present study was to examine sensitization to the seizurogenic effects of subconvulsant doses of both CE and cocaine. In addition, the potentially clinically relevant interactive sensitization effects between cocaine and cocaethylene were assessed.

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METHOD

A total of 58 (29 male and 29 female) experimentally naive HS mice served as subjects. Animals were individually housed in 27 × 18 × 12 cm clear Plexiglas cages bedded with aspen shavings and equipped with wire tops. Animals were maintained in a climate-controlled Vivarium on a 12 L : 12 D cycle with light onset at 0600 h. Food and water were available ad lib. All mice were 25–30 days of age at the beginning of experimentation.

Prior to experimentation, animals were randomly assigned to one of four experimental conditions (n = 10 mice per condition with an equal number of males and females) or one of three control conditions (n = 6 per condition with an equal number of males and females represented). Two experimental conditions consisted of animals that received 48 mg/kg of cocaine injected once per day for 4 successive days. On the fifth day, one of these groups received another injection of 48 mg/kg cocaine (COC/COC), the other group was administered 32 mg/kg cocaethylene (COC/CE). Animals in the remaining two experimental conditions received 32 mg/kg cocaethylene injected on days 1–4 followed by administration of either 32 mg/kg cocaethylene (CE/CE) or 48 mg/kg cocaine (CE/COC) on day 5. Animals in the three control conditions received saline vehicle injections on days 1–4 and either saline (SAL/SAL), 48 mg/kg cocaine (SAL/COC), or 32 mg/kg CE (SAL/CE) administered on day 5.

Procedure

All procedures were conducted between 1200 and 1800 h. Animals were transported, in their home cages, from the Vivarium holding room to an immediately adjacent area. Animals were weighed and briefly placed into a clear Plexiglas cage identical to the home cage but without bedding. Animals were then injected with their designated drug/vehicle, replaced into the Plexiglas cage, and a timer was started. Animals were monitored for 120 min for indications of seizure or lethality. Death was defined as the absence of respiration for a period of greater of 30 s.

Following the observation period, animals were returned to the home cage in the holding area. This procedural regimen occurred each day for a period of 5 days.

Drugs

Animals in designated groups were administered either 48 mg/kg cocaine hydrochloride (NIDA) or 32 mg/kg cocaethylene fumarate (Sigma Chemical Co.). Drug doses were calculated as base and dissolved fresh daily in saline. Dosages were chosen based on earlier, unpublished work from this laboratory indicating that these doses produce minimal seizurogenic activity in HS mice. All injections were delivered intraperitoneally in a constant volume of 5 ml/kg body weight.

RESULTS

Within each group there were no differences in frequency of seizures across days between males and females. Therefore, the frequency data obtained were not analyzed for sex differences; group frequencies, as depicted in Table 1, were also not subdivided according to sex.

Frequency of seizures on day 1 vs. day 5 was analyzed for each of the four groups using the McNemar test (18) for related samples. The single deviation from this analysis occurred in the case of the CE/COC group. Three animals in this group died on day 4, apparently from toxic effects related to CE administration. Because this reduction in the number of subjects invalidated the McNemar test (18), data obtained from the nonsurviving subjects were discarded and day 1 vs. day 5 data from the remaining seven animals were analyzed using the Binomial test for small samples.

These analyses indicated a significant increase in the frequency of cocaine- (COC/COC) and cocaethylene (CE/CE)-induced status epilepticus between day 1 and day 5 (χ^2 = 7.11, $p < 0.05$), suggesting that, like cocaine, cocaethylene is capable of kindling status epilepticus when nonseizurogenic doses are administered for several days. Furthermore, animals that received cocaine administration for 4 days followed by cocaethylene injections on day 5 also exhibited an increase in seizure frequency on day 5 (χ^2 = 7.11, $p < 0.05$), indicating potential cross-sensitivity between cocaine and cocaethylene (Table 1). However, animals given repeated cocaethylene exposure prior to cocaine injection (CE/COC) failed to display a significant increase in status epilepticus between day 1 and day 5 (p = 0.50). Nonetheless, a tendency toward cross-generalization is apparent in that administration of cocaine following cocaethylene exposure produced seizures in four of the seven animals (see Table 1).

With respect to the kindling of tonic-clonic seizures, data indicated that only animals in the COC/COC groups displayed a significant increase in the number of tonic-clonic seizures observed on day 1 vs. day 5 (χ^2 = 7.11, $p < 0.05$). Last, saline control groups produced no observable behavioral change except for one SAL/COC animal displaying tonic-clonic seizure on day 5 (data not shown).

DISCUSSION

Studies examining the kindling effects of cocaine in mice have indicated that different inbred strains of animals display marked differences in their response to subconvulsant doses of cocaine (8,9). The HS mice are a genetically heterogenous stock produced by intercrossing eight inbred mouse lines, including the C57 strain. It has been suggested that employing this HS line may help to reduce the reportedly significant differences in acute sensitivity to cocaine convulsions between mouse strains (10). The present data with HS mice indicate that repeated daily administrations of 48 mg/kg cocaine produced seizures in 90% of the mice on day 5. This peak closely parallels data obtained by Marley et al. (9) in C57 mice in that this line, unlike others, displayed a rapid induction of seizure activity when 50 mg/kg cocaine was administered daily; specifically, 0% of the C57 mice responding with seizures on day 1, 0% on day 2; 30% on day 3 and 80% on day 4, culminating with 90% responsiveness on day 5. While not a perfect correspondence, the current data obtained with HS mice closely mirrors the effects observed with a similar dose in C57 animals, suggesting that the propensity for cocaine-induced kindling in HS animals may be similar to that of C57 mice. However, it should be noted that continued administration of cocaine on day 6–10 in C57 mice produced marked desensitization (10–0% responding) to the seizurogenic effects of cocaine (8,9). Whether this rapid desensitization effect is evident in the HS strain of mice remains to be determined.

Repeated administration of cocaethylene produced a kindling pattern very similar to that of cocaine (90% responding by day 5) with respect to status epilepticus. As has been previously reported (11), cocaethylene does not produce tonic-clonic seizure activity with a single injection of a convulsant

TABLE 1

FREQUENCY OF STATUS-EPILEPTICUS AND TONIC-CLONIC SEIZURES IN MICE

	Day 1	Day 2	Day 3	Day 4	Day 5
COC/COC (n = 10)					
Tonic-clonic	2	3	7	8	9
Status-epilepticus	0	3	6	8	9
CE/CE (n = 10)					
Tonic-clonic	0	0	0	0	0
Status-epilepticus	0	2	3	7	9
COC/CE (n = 10)					
Tonic-clonic	1	4	6	9	5
Status-epilepticus	0	3	5	8	9
CE/COC (n = 7)					
Tonic-clonic	0	0	0	0	4
Status-epilepticus	0	1	2	4	4

Animals were administered 48 mg/kg cocaine or 32 mg/kg cocaethylene once per day over four days. On Day 5, animals were injected with either the same or alternative psychostimulant.

dose. The present findings reconfirm this effect because a subconvulsive dose of cocaine was able to kindle both tonic-clonic seizures and status epilepticus, whereas cocaethylene-treated animals displayed only the kindling of status epilepticus with no evidence of sensitized tonic-clonic seizure induction.

It may be possible that seizure activity occurs along an intensity continuum and the observed lack of tonic-clonic activity in cocaethylene-treated animals simply may reflect more severe phase toxicity. Based on the data presented in the current study, it is difficult to adjudge the possible relationship between tonic-clonic seizures and status epilepticus. Clearly, each seizure type is characterized by a different cluster of behavioral manifestations (11). Additionally, cocaine and cocaethylene produce markedly different seizure profiles (i.e., the absence of tonic-clonic events with cocaethylene administration). While the notion of a severity continuum with respect to seizure activity is intriguing, several studies from our laboratory have provided evidence to suggest that the two seizure events may be unrelated. Fenfluramine administration results in a decreased onset latency (with no increase in frequency) of cocaine-induced tonic-clonic seizures. Yet cocaine-induced status epilepticus occurs more rapidly and with greater frequency following fenfluramine treatment, suggesting that 5-HT agonism may differentially promote induction of status epilepticus relative to tonic-clonic activity (17). Furthermore, this enhancement in status epilepticus does not produce increased lethality. Additionally, the instance of cocaine-induced status epilepticus can be completely blocked by the administration of the 5-$HT_{2/1c}$ antagonist cinanserin, while the frequency of tonic-clonic seizures remains unaltered, supporting the notion that the two seizure types may be distinctive (17). Finally, cocaethylene does produce status epilepticus but the intensity and duration of this effect is no different from that produced by cocaine (unpublished observation). Furthermore, status epilepticus is not highly predictive of cocaethylene-induced lethality. The most predictive premorbid behavior is a diaphragmic spasm (rhythmic hard contractions of the diaphragm (11). In our laboratory, all animals displaying this behavior have succumbed to the lethal effects of cocaethylene.

Thus, the current data suggest that cocaine and cocaethylene are quite similar in their ability to produce kindling of status epilepticus but a fundamental difference in drug action may be inferred by the 2 drugs' consequent differential effects on the kindling of tonic-clonic activity. This difference in drug action is also addressed by the data obtained from the cross-sensitization groups. Animals that received cocaethylene after 4 days of cocaine administration displayed cross-sensitization as 90% of them display status epilepticus on day 5. However, animals given repeated cocaethylene exposure prior to cocaine injection (CE/COC) failed to display a significant increase in status epilepticus between day 1 and day 5 (p = 0.50). Nonetheless, a tendency toward cross-generalization is apparent in that administration of cocaine following cocaethylene exposure produced seizures in four of the seven animals. If there were no cross-sensitivity between the two drugs, the expected frequency should have been similar to that observed following initial administration of the cocaine challenge dose (see Table 1).

These data indicate that subconvulsant doses of cocaine may produce sensitization to the effects of cocaethylene-induced status epilepticus. However, any conclusions as to the validity of the symmetrical finding (i.e., that repeated subthreshold doses of cocaethylene may produce sensitization of cocaine-induced status epilepticus) must be tempered in light of the small number of subjects employed in the latter manipulation.

With respect to tonic-clonic seizure activity, neither the COC/CE nor the CE/COC group displayed a significant cross-sensitization effect. However, it should be noted that 50% of the COC/CE animals displayed tonic-clonic seizures upon administration of cocaethylene on day 5. Indeed, this is the only incidence in which cocaethylene has produced tonic-clonic seizures in HS mice (11,16). Thus, it may be possible that repeated subconvulsant doses of cocaine produce an alteration in CNS excitability that allows for the expression of tonic-clonic seizure characteristics upon acute administration of cocaethylene. Alternatively, given the reduction in tonic-

clonic seizure frequency (from 90% on day 4 with cocaine to 50% on day 5 with cocaethylene) it is plausible that cocaethylene (like its parent drug ethanol) has some anticonvulsant properties with respect to tonic-clonic activity. Additionally, over the course of 4 days of exposure to cocaethylene, animals exhibited no tonic-clonic seizures yet the challenge dose of cocaine on day 5 produced a tonic-clonic seizure frequency greater than that produced by initial cocaine exposure, suggestive of an interactive effect.

A possible explanation for these effects may reside in the differential affinity of cocaine and cocaethylene for serotonergic receptors and the potential serotonergic mediation of the induced seizures observed. Cocaethylene has been reported to be less potent than cocaine in regard to its effects on serotonergic neuronal systems (1). Furthermore, cocaine's blockade of presynaptic serotonergic transport has been linked to its seizurogenic effects (15,17). Based on this, the process of cocaine sensitization may alter serotonergic transporter systems or postsynaptic receptors such that cocaethylene could produce cross-sensitization even with low serotonin affinity. However, this scenario cannot account for the effects of cocaine administration following cocaethylene pretreatment. Clearly, further research as to the potential mechanisms of cross sensitization between these two drugs is warranted. In light of the observation that cocaine, with its rapid time course of action, is intermittently and repeatedly administered by human abusers, the sensitization effects seen with these repeated low doses may be of clinical interest. With the next administration of a previously innocuous dose, the possibility of a more severe reaction exists. The mechanisms of action by which cocaine and its ethanol-induced metabolite cocaethylene produce these heightened effects may be mediated by differential actions upon neurotransmitters. Additional research will allow for evidence as to possible mechanisms for sensitization by psychostimulants.

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