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Serotonergic Mediation of Cocaine Seizures in Mice

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SCHECHTER, M. D. AND S. M. MEEHAN. *Serotonergic mediation of cocaine seizures in mice.* PHARMACOL BIOCHEM BEHAV **51**(2/3) 313-316, 1995.—We used genetically heterogeneous HS mice to investigate the effects of drugs that alter brain concentrations of serotonin on cocaine-induced convulsions and lethality. The racemer of fenfluramine, which increases synaptic serotonin, was coadministered with a dose (60 mg/kg, intraperitoneally) of cocaine that does not produce status epilepticus or death. This drug combination significantly increased the occurrence and decreased the time of onset of status epilepticus, but did not affect lethality. Likewise, 2.5 mg/kg of the L-isomer, but not the D-isomer, of fenfluramine increased the occurrence of status epilepticus. Neither isomer effected lethality. When 2.5 mg/kg cinanserin, a drug that antagonizes postsynaptic serotonergic receptors, was coadministered with a higher (95 mg/kg) dose of cocaine, the time of onset of status epilepticus was significantly increased, whereas lethality was reduced. The results are discussed in light of the action of cocaine upon serotonin neurons and the relationship between seizurogenic activity and cocaine-induced lethality.

Cocaine Serotonin Seizures Fenfluramine Cinanserin Mice

GENERALIZED tonic-clonic seizures occur infrequently during the therapeutic use of drugs but may be considered a relatively common neurologic complication of recreational drug use. Cocaine has been identified as the leading cause of convulsions in drug abusers, and episodes have been reported in both first-time and chronic users (1). Epidemiologic data indicate that the relative frequency of seizures among cocaine abusers ranges from 2% to as much as 27%, and the frequency of seizures based on emergency room presentations for cocaine toxicity ranges from 2.3–8.4% (20). Although the prevalence of death from cocaine overdose is thought to be low but growing, the contribution of seizures to that mortality has been well established (2). Because the major organ systems at risk in cocaine toxicity in humans involve the central nervous and cardiopulmonary systems, animal models have been used to examine each. This study emphasizes animal models documenting the seizure toxicity of cocaine (3,5,9,22,28). One of these reports (9) suggests that there is a dissociation between cocaine-induced seizures and death, and that each of these entities is mediated by distinct and different neuronal mechanisms. Furthermore, a second report (21) from this same investigator suggested that it is cocaine's effects on dopamine, σ-, and/or muscarinic receptors that produces lethality, whereas seizures may in fact be related to cocaine's interaction with presynaptic serotonergic transporters.

In a series of experiments to determine the premorbid behaviors (18), as well as the lethality of coadministration of cocaine and ethanol (23), analysis of data established that a 60-mg/kg dose of cocaine produced some seizurogenic activity but no deaths, whereas a higher (95 mg/kg) cocaine dose invariably caused convulsions and deaths in male and female HS-strain mice. To further test the serotonergic mediation of the seizure-producing properties of cocaine, the selective 5-$HT_{2/1c}$ antagonist cinanserin (27) was coadministered with a convulsive dose of cocaine, whereas the racemer and isomers of the indirect serotonergic agonist fenfluramine (6) was administered with the lower dose. The occurrence and time of onset of tonic-clonic seizure, status epilepticus, and death were then determined in male and female HS mice. This strain has been developed by the Institute of Behavioral Genetics at the University of Colorado at Boulder, where the foundation population were genetically heterogeneous stock bred by intercrossing eight inbred mouse lines (17).

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METHOD

Subjects and Procedure

A total of 140 (70 male and 70 female) experimentally naive HS mice were used to determine the effects of cocaine with and without coadministration of serotonergic agents in the production of convulsive activities and lethality. Animals were individually housed in 29 × 18 × 12-cm clear Plexiglas cages with aspen shavings as bedding and covered by wire tops. These cages were kept in a climate-controlled colony room in the Vivarium facility set on a 12 L : 12 D cycle with lights on at 0600 h. Food and water were continually available. The mice were 28–30 days of age at testing.

All procedures were conducted between 1200 and 1800 h. The animals were transported in their home cages from the colony room to an adjacent experimental area. Each animal was randomly assigned a treatment, weighed, and briefly placed into a clear Plexiglas cage identical to its home cage but without bedding. They were then injected, in their contralateral lower quadrants, with either (0.9%) saline or a serotonergically active compound and replaced into the Plexiglas cage. Thirty minutes later, animals were injected with cocaine and replaced into the Plexiglas cage, and a timer was set. Animals were monitored over a 120-min period for indications of toxicity and lethality. A tonic-clonic seizure was defined as being characterized by extension of one or both hind limbs at an angle > 90° accompanied by gaping (mouth open with head raised 30–45° from horizontal); status epilepticus was defined as violent, motoric seizures during which the mouse "rebounded" off the bottom and sides of the test cage; death was operationally defined as the absence of respiration for a period > 30 s. Those mice surviving beyond the 120-min observational period remained in the procedure cage and were subsequently returned to the colony room with food and water available. As has been reported previously in the literature (10), no deaths were observed after 30 min. Nonetheless, the animals were reexamined at 16 and 24 h, after which time any surviving animals were euthanized by CO inhalation.

Drugs, Dosage Regimens, and Statistical Analysis

Previous evidence (23) indicated that intraperitoneal (IP) administration of 60 mg/kg cocaine produces tonic-clonic seizures in the majority of HS mice but no lethality, whereas 95 mg/kg cocaine produces slightly greater incidences of tonic-clonic seizures but also produces status epilepticus and > 50% lethality. The lower dose was coadministered with 2.5 mg/kg D,L-fenfluramine, as well as a similar dose of both D- and L-fenfluramine. The 95-mg/kg cocaine dose was coadministered with 2.5 and 5.0 mg/kg cinanserin. Each drug was readily dissolved in 0.9% saline immediately before use and administered IP in a 5-ml/kg volume. Cinanserin HCl was a gift from E.R. Squibb and Sons, Inc. (New Brunswick, NJ); D,L-fenfluramine HCl was purchased from Sigma Chemical Co. (St. Louis, MO); and the D- and L-isomers of fenfluramine HCl were donated by Servier Laboratories (Paris, France). χ^2 analysis (24) was used to analyze the frequency of occurrence of seizures and death. Behavioral latencies were analyzed with one-way ANOVAs followed by planned comparisons.

RESULTS

Results in male and female mice were shown to be similar. Therefore, data from both sexes were combined to allow for n = 20 in each treatment group. Figure 1 depicts data pertaining to the frequency of seizures and death; Table 1 presents latencies to seizures and death.

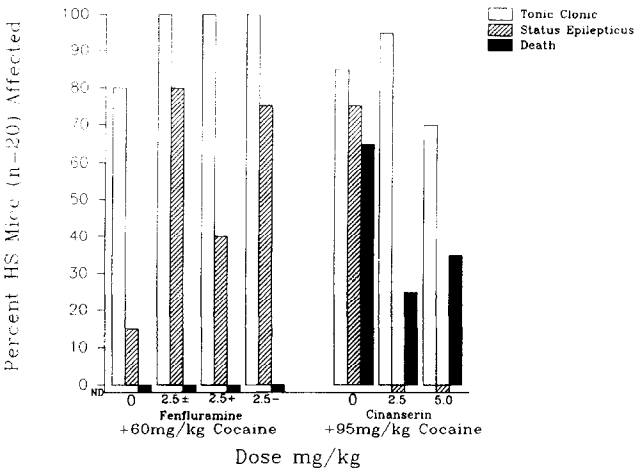

FIG. 1. Percent of HS mice (n = 10 males, 10 females) experiencing tonic-clonic seizures, status epilepticus, or death. ND, Not detected. Cotreatments with cinanserin and fenfluramine racemer-isomers were administered intraperitoneally on contralateral sides 30 min before cocaine, and mice were observed for a 120-min period.

One-way ANOVA conducted on latencies to tonic-clonic seizure in animals treated with 60 mg/kg cocaine alone, in combination with D,L-fenfluramine or its isomers, revealed a significant effect [$F(3, 72) = 8.59$, $p < 0.05$]. Subsequent analysis indicated that D,L-fenfluramine given in combination with 60 mg/kg cocaine significantly decreased the onset time of tonic-clonic seizures relative to that seen with cocaine administration alone [$F(1, 72) = 12.65$, $p < 0.05$]. Similar effects were also observed for both D- and L-fenfluramine [$F(1, 72) = 24.55$ and 7.05, $p < 0.05$, respectively; Table 1]. However, analysis of frequency of tonic-clonic seizures indicated that fenfluramine in any form failed to enhance seizure occurrence ($\chi^2 = 2.5$, NS) (Fig. 1).

Analysis of latency to onset of status epilepticus also indicated that fenfluramine treatment in conjunction with cocaine produced significant effects [$F(3, 39) = 7.51$, $p < 0.05$]. Comparisons showed that D,L-fenfluramine, D-fenfluramine, or L-fenfluramine pretreatment hastened the onset of status epilepticus relative to subjects receiving cocaine alone [$F(1, 39) = 19.72$, 18.79, and 12.84, $p < 0.05$, respectively; Table 1]. Analysis of frequency indicated that whereas both D,L-fenfluramine and L-fenfluramine enhanced the frequency of occurrence of status epilepticus ($\chi^2 = 14.44$ and 12.22), no effect was observed for D-fenfluramine ($\chi^2 = 2.00$, NS), suggesting that this isomer may be less effective in promoting seizure induction when give in combination with cocaine.

In light of these significant effects of fenfluramine on seizure activity, it should be noted that none of the fenfluramine treatments enhanced cocaine lethality (Fig. 1).

Analyses conducted on data from animals that received 95 mg/kg of cocaine alone, or in combination with 2.5 or 5.0 mg/kg of cinanserin, revealed that the latency to tonic-clonic seizure was significantly altered by cinanserin pretreatment [$F(3, 34) = 14.29$, $p < 0.05$]. As can be seen in Table 1, cinanserin at 2.5 and 5.0 mg/kg significantly lengthened the time to onset of seizure [$F(1, 34) = 9.92$ and 11.90, $p < 0.05$, respectively]. However, cinanserin treatment had no effect on

TABLE 1

MEANS (±SE) FOR BEHAVIOR LATENCIES ASSOCIATED WITH 60 OR 95 mg/kg COCAINE ADMINISTERED ALONE OR IN COMBINATION WITH SPECIFIED SEROTONERGIC AGENTS

Drug (mg/kg)	Tonic Clonic	Status Epilepticus	Death
60 Cocaine	251.94 (23.6)	307.50 (43.9)	ND
60 Cocaine + 2.5 D,L-fenfluramine	181.30 (10.3)	186.34 (12.8)	ND
60 Cocaine + 2.5 D-fenfluramine	156.45 (6.4)	178.00 (16.0)	ND
60 Cocaine + 2.5 L-fenfluramine	197.55 (12.7)	209.13 (8.7)	ND
95 Cocaine	97.53 (7.5)	107.73 (11.9)	345.16 (47.8)
95 Cocaine + 2.5 cinanserin	159.26 (8.7)	ND	648.00 (97.48)
95 Cocaine + 5.0 cinanserin	220.6 (9.2)	ND	683.61 (51.12)

ND, Not detected.

the frequency of seizures (χ^2 = 0.28 and 1.2, NS). With respect to the incidence of status epilepticus, both doses of cinanserin abolished the incidence of status epilepticus (Table 1 and Fig. 1).

Analysis of latencies until death revealed a significant effect [$F(3, 15) = 10.22, p < 0.05$], with both 2.5 and 5.0 mg/kg of cinanserin prolonging the time until death following cocaine administration [$F(1, 15) = 9.92$ and 11.90, $p < 0.05$]. In terms of frequency of deaths, 2.5 mg/kg of cinanserin significantly reduced cocaine-induced lethality (χ^2 = 4.9); however, the higher dose (5.0 mg/kg) failed to alter the frequency of death.

DISCUSSION

The mechanism(s) by which a large dose of cocaine induces seizures and/or lethality is complex. Nevertheless, a recent study indicated that potency at the serotonergic transporter is the best single predictor of seizure activity for cocaine as well as cocaine-related drugs (21). In addition, the assumption that cocaine-related lethality is causally linked to these seizures may in turn infer that the lethal effects of cocaine are produced by serotonergic mechanisms. However, other distinct biochemical mechanisms may be mediating cocaine's effects [e.g., binding to dopamine transporters (9,10)]. Results of the present study would suggest that increasing the concentration of serotonin at the synapse by increased release and/or reuptake inhibition, as posited for the mechanism of fenfluramine action (8), may be related to seizure-inducing effects of cocaine. However, these data also indicate that seizurogenic effects may be unrelated to cocaine lethality. Fenfluramine, in its racemer and isomer forms, decreased the latency of onset for cocaine-induced tonic-clonic seizures, but failed to alter the frequency of seizures, suggesting that its effects on 5-HT levels hastened tonic-clonic seizure induction but did not promote it.

With respect to status epilepticus, latency to cocaine-induced seizure was decreased by all fenfluramine pretreatments. In addition, both D,L- and L-fenfluramine increased seizure frequency. This pattern suggests that unlike tonic clonic seizures, cocaine-induced episodes of status epilepticus can be both hastened and promoted by altering 5-HT activity. Given the fact that fenfluramine has no neuroprotective activity upon seizure severity when administered alone (15), it is suggested that the effects of cocaine upon serotonin may indeed affect seizure activity.

In light of these effects, the failure of fenfluramine pretreatment to alter cocaine-induced lethality is particularly interesting. The current data suggest that the lethal effects of cocaine are unrelated to its seizurogenic properties, as has been previously shown in other animal seizure models (4, 13,16).

Administration of the 5-$HT_{2/1c}$ antagonist cinanserin increased the latency of tonic-clonic seizures but did not affect the frequency of seizures, once again suggesting that alteration of 5-HT does not produce changes in cocaine-related tonic-clonic seizure induction. However, cinanserin treatment completely eliminated the incidence of status epilepticus, indicating that this type of seizure is in some way different from tonic-clonic seizures in that antagonism of 5-$HT_{2/1c}$ receptors appears to have an effect on the induction of status epilepticus by cocaine. Other neuronal systems, such as the glutamatergic (12) or adrenoreceptors (7), may also have a contributory role in the convulsive activity and lethality produced by cocaine.

Both doses of cinanserin prolonged the time until death, but only the low (2.5-mg/kg) dose actually decreased the frequency of death. Given that both doses significantly altered the seizurogenic responses to cocaine, the failure of the higher dose to decrease the incidence of death tentatively supports the previously mentioned notion that cocaine's lethal effects are unrelated to seizure production.

Seizures, which are frequently recognized manifestations of acute cocaine use, can occur not only in first-time users but also in chronic drug users, regardless of age (1). This side-effect may or may not be directly linked to the increasing number of cocaine-related deaths being reported in humans (14). In addition, numerous drugs such as diazepam, which are clinically useful for seizure disorders of any etiology, have been shown to be effective in reversing seizures produced by acute overdose of cocaine (25). The present study may help evidence the central neurotransmitter system, viz., serotonergic neuronal pathways, that function differentially in the mechanism of cocaine seizures; in turn, this evidence may allow for the development of more specific agents to counteract the increasing prevalence of cocaine seizures (14).

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