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BRIEF COMMUNICATION

The Lethal Effects of Ethanol and Cocaine and Their Combination in Mice: Implications for Cocaethylene Formation

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SCHECHTER, M. D. AND S. M. MEEHAN. *The lethal effects of ethanol and cocaine and their combination in mice: Implications for cocaethylene formation.* PHARMACOL BIOCHEM BEHAV 52(1) 245–248, 1995.—The HS line of mice was used to determine the LD_{50} values for cocaine and ethanol, as well as for cocaethylene, the enzymatic product of their coadministration. The LD_{50} of cocaethylene was found to be significantly lower than that of cocaine, and both were more potent in their lethality than ethanol. When a low-lethality dose of cocaine was administered with a nonlethal dose of ethanol, the result was a significant increase in the prevalence of lethality. Thus, the lethal effects of the dose of cocaine used were increased by the dose of ethanol administered such that the two drugs in combination were equipotent to cocaethylene. The results are discussed in light of the ability of the liver, via transestification, to rapidly form cocaethylene from cocaine in addition to ethanol's ability to decrease the catabolism of cocaine. Thus, the possibility exists that the increased lethality observed is produced by both the production of the more lethal cocaethylene and sustained levels of cocaine.

Cocaine Ethanol Cocaethylene Lethality Mice

COCAETHYLENE is produced via a human liver carboxylesterase reaction when cocaine is administered in conjunction with ethanol (5). Cocaethylene has a pharmacologic profile similar to that of cocaine in that it blocks presynaptic transport of dopamine, resulting in increased synaptic levels and enhanced postsynaptic stimulation (10); this increase in brain dopamine may in turn result in a subjective state of euphoria. Although cocaethylene appears to be equipotent with cocaine in terms of its effect on dopamine transport (10), the compound has been reported to be less potent in regard to its effects on serotonergic neuronal systems (3), and this reduced efficacy of cocaethylene may suggest why the substance appears to be even more euphorigenic than cocaine (13). This enhancement of the subjective experience may provide an indication as to why coingestion of alcoholic beverages and cocaine is a prevalent combination amongst polydrug users and abusers (9).

In animal studies, cocaethylene has been shown to be more potent than cocaine in producing lethality in rodents (11,12). In addition, ethanol potentiates acute cocaine toxicity in rats (17) and hepatotoxicity in mice (1,19) as well as humans (13). Finally, cardiac toxicity in humans appears to be enhanced by the combination of ethanol and cocaine when compared to that observed when either drug is taken alone (13). Postmortem analysis for the presence of cocaethylene in the blood and brain tissue of individuals self-administering cocaine and ethanol (23) has led to speculation on the mediating role of cocaethylene in cases of apparent cocaine toxicity leading to sudden death (11). However, this position is not without controversy. Recent studies examining combined toxicity of ethanol and cocaine in both baboons (8) and humans (7) have indicated that the enhanced toxicity of these two drugs in combination may not result from the effects of cocaethylene formation, but rather, may be related to an addi-

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tive effect resulting from the direct actions of cocaine and ethanol.

The potential lethal actions of cocaine itself are well known (26), and ethanol may have a potentiating effect on these mechanisms, because it is known to alter in vivo drug effects by producing changes in drug metabolism and pharmacokinetics (21). Such dispositional effects may prolong blood and brain levels of cocaine. In fact, ethanol intoxication has been shown to increase plasma levels of cocaine (18), and chronic ethanol exposure has been shown to elicit an increase in cocaine brain-plasma ratios in rats, although there was no similar increase in the cocaine concentration in plasma, heart, or lung (25). In contrast, Fowler et al. (7) indicated that ethanol administered in conjunction with radiolabeled cocaine failed to produce any alterations in uptake distributional clearance of cocaine in the brain or hearts of human volunteers. Thus, current information regarding the effects of ethanol on the pharmacokinetics of cocaine remains unclear.

The aim of this study was therefore twofold; first, to establish a dose-response lethality profile for ethanol, cocaine, and cocaethylene; the results of employing the latter two drugs were sought to replicate the recent report of Hearn et al. (11). The second aim was to examine the lethality of ethanol and cocaine when coadministered, in an effort to compare the lethality produced by administration of the cocaethylene metabolite itself with that seen after administration of the antecedent combination of ethanol plus cocaine.

METHOD

Subjects and Procedure

A total of 270 (135 male and 135 female) experimentally naive HS mice were used to generate the LD_{50} values for ethanol, cocaine, and cocaethylene, as well as combinations of ethanol and cocaine. The protocol (no. 93-057/19) was reviewed and approved by the College of Medicine's Institutional Animal Care and Use Committee. The HS strain has been developed by the Institute of Behavioral Genetics at the University of Colorado at Boulder, where the foundation population was genetically heterogeneous stock (HS) produced by intercrossing eight inbred mouse lines (16). These may help to reduce the reported (15) significant differences in the acute sensitivity to cocaine convulsions between mouse strains. Animals were individually housed in 29 × 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.

All procedures were conducted between 1200 and 1800 h. Animals were transported in their home cages from the Vivarium holding room to an adjacent area. Each animal was randomly assigned a drug dosage, weighed, and then briefly placed into a clear Plexiglas cage identical to the home cage but without bedding. Animals were then injected and replaced into the Plexiglas cage, and a timer was started. Animals were monitored over a 120-min period for indications of toxicity and lethality. Death was operationally defined as the absence of respiration for a period > 30 s. Animals surviving beyond the 120-min observation period remained in the procedure cage and were returned to the holding area with food and water available ad lib. As reported previously (11), no deaths were observed in mice after 30 min. They were examined again at 16 and 24 h. After 24 h, all animals were euthanized by CO inhalation.

Determination of LD_{50} for Ethanol, Cocaine, and Cocaethylene

Each animal in the ethanol group received two concurrent intraperitoneal (IP) injections. The first injection consisted of (20% w/v) ethanol at doses of 6.0, 8.0, or 10.0 g/kg delivered in a 10-ml/kg volume. The second injection, delivered immediately after the first but into the contralateral side, consisted of 0.9% saline in a 5-ml/kg volume.

Animals in the cocaine and cocaethylene groups also received two concurrent injections. The first injection consisted of a saline injection delivered IP in a 10-ml/kg volume followed by a second IP injection consisting of either cocaine hydrochloride (NIDA) in one of four doses (60, 75, 95, or 120 mg/kg) or cocaethylene fumarate (Sigma Chemical Co., St. Louis, MO) in one of four doses (48, 60, 75, or 95 mg/kg). The second administration of drug dose was calculated as base, dissolved fresh daily in saline and delivered in a constant volume of 5 ml/kg into the contralateral lower quadrant.

Determination of LD_{50} of Ethanol and Cocaine in Combination

Animals in this subsequent study were divided into two groups with both groups receiving two concurrent injections. One group of males and females received a 6.0-g/kg (LD_{50} as determined above) dose of ethanol followed by either 48, 60, or 75 mg/kg cocaine hydrochloride. The second group was administered either 1.5, 3.0, or 6.0 g/kg ethanol followed by a 75-mg/kg dose of cocaine hydrochloride. This dose of cocaine was selected for coadministration because it produced a minor degree of lethality, thus allowing an examination of the potential augmenting or ameliorative effects of ethanol on cocaine-induced mortality. Drug preparation, administration volumes, injection sites, and procedures were consistent with those described earlier.

Statistical Analysis

LD_{50} values with 95% confidence limits, as well as potency ratios, were derived using a computerized version of the Litchfield-Wilcoxon analysis (24).

RESULTS

Cocaethylene was shown to produce an LD_{50} (95% confidence limits) of 67.21 (54.78–82.46) mg/kg in males and 64.27 (55.89–73.91) mg/kg in females. As can be seen in Fig. 1, cocaethylene was significantly ($p < 0.05$) more potent (potency ratio = 0.68) than cocaine in producing lethality, with LD_{50} values for cocaine calculated as 101.55 (88.31–116.76) mg/kg in males and 90.0 (79.82–101.48) mg/kg in females. Although females appeared to be slightly more sensitive to the lethal effects of cocaine, the potency ratio (1.84) between males and females was not significant (24). Ethanol was shown to be dramatically less potent with respect to lethality than either cocaethylene or cocaine, with an LD_{50} of 9.71 (8.38–11.27) g/kg in males and 9.45 (8.45–10.49) g/kg in females. The dose-response results with varying doses of cocaethylene and cocaine closely replicate previous results (11).

Varying the dose of ethanol that was coadministered with 75 mg/kg cocaine produced an enhanced degree of lethality (Fig. 2). LD_{50} values for ethanol administered with 75 mg/kg cocaine were lower than those observed with ethanol administered alone as they were reduced to 3.46 (2.08–5.75) g/kg in males and 1.88 (1.25–2.82) g/kg in females. Analysis of the potency ratio in both genders of mice when ethanol was ad-

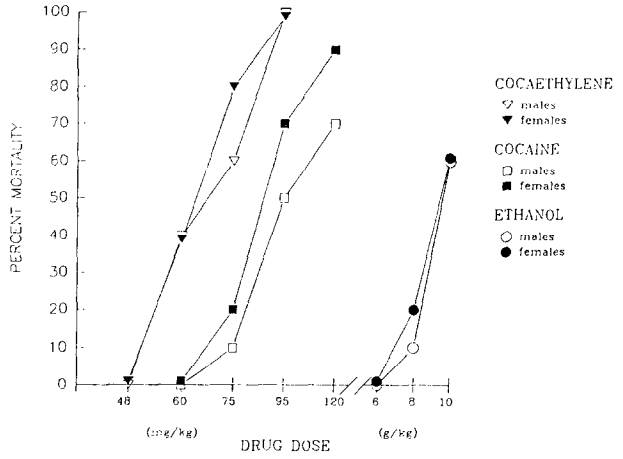

FIG. 1. Lethality of cocaethylene, cocaine, or ethanol in male and female mice. Each data point represents n = 10 males and 10 females per dose except for cocaethylene, where n = 5 male and 5 female mice.

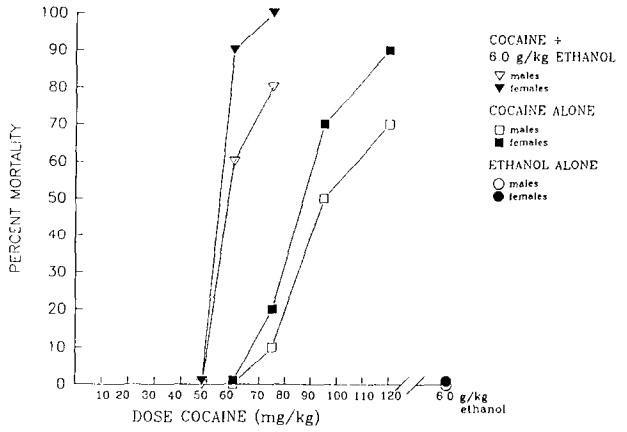

FIG. 3. Lethality of cocaine administered alone or in lower dose combinations with 6.0 g/kg ethanol in male and female mice. Data points for ethanol and cocaine alone are from Fig. 1. Data points for 6.0 ethanol plus 75 cocaine are from Fig. 2.

ministered alone vs. ethanol plus cocaine results indicated that the lethal potency of ethanol was significantly enhanced when it was administered in conjunction with 75 mg/kg cocaine.

Similarly, the coadministration of varying doses of cocaine with 6.0 g/kg ethanol resulted in an enhanced lethality of cocaine (Fig. 3). The LD_{50} values for cocaine were reduced (from 101.55) to 60.92 (50.88–72.95) mg/kg in males and (from 90.0) to 51.16 (45.43–57.61) mg/kg in females. Although females appeared to be slightly more sensitive to the combined effects of ethanol and cocaine, the potency ratio for the drug combination indicated no significant difference between males and females. Further analysis indicated that the combination of 6.0 g/kg ethanol and cocaine was more potent in producing lethality than cocaine alone, whereas the two drug combination was equipotent to cocaethylene in the production of lethality.

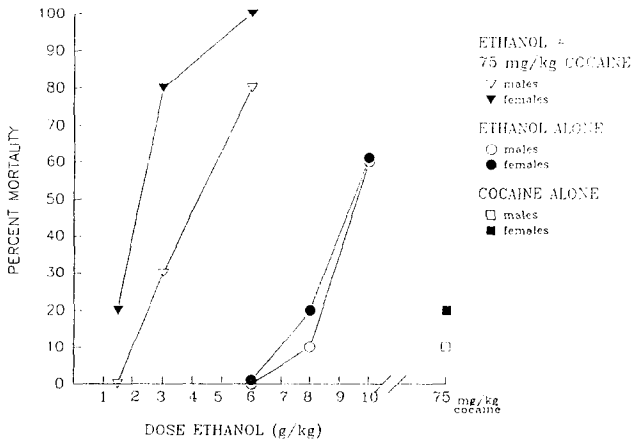

FIG. 2. Lethality of 6, 8, and 10 g/kg ethanol administered alone or in lower dose combinations with 75 mg/kg cocaine in male and female mice. Data points for ethanol and cocaine alone are from Fig. 1.

DISCUSSION

A recent report (11) using male and female CD-1 outbred Swiss Webster mice, compared the acute toxicity of cocaethylene with cocaine and found the LD_{50} values for the former to be 60.7 and 63.8 mg/kg in female and male mice, respectively, whereas the latter LD_{50} value was 93.0 mg/kg in both sexes. The present study employed the HS strain of mouse, which has the advantage of being heterogeneously bred from eight different lines of mice (16), and the results indicated similar LD_{50} values for both cocaethylene and cocaine. Thus, both studies showed that cocaethylene is more potent than cocaine in producing lethality in mice. In addition to this replication of results in the HS line of mice, the LD_{50} value for ethanol was determined in both genders and was also similar to the value most often reported in the literature for other lines of mice (6,14,22).

When a dose of cocaine (75 mg/kg) that produced a low level of lethality (i.e., 10% mortality in males and 20% mortality in females) was coadministered with a dose of ethanol (6 g/kg) that produced no lethality, the resulting lethality was 100% in females and 80% in males (Figs. 2 and 3). This potentiation of cocaine lethality by ethanol in mice is congruent with previously reported findings in rats (17). A plausible explanation for this observed potentiation resides in the very rapid (i.e., within 2.5 min) peak of hepatic concentration of the ethyl ester of cocaine (i.e., cocaethylene) reached after the administration of both drugs in mice (2). This rapid formation of cocaethylene and its apparent increased toxicity (Fig. 1) may help to explain the increased lethality produced by the coadministration of nonlethal doses of ethanol and cocaine. However, this conclusion must await analysis of cocaethylene concentrations to indicate the amounts produced and thereby contributing to lethality.

In addition to the rapid formation of cocaethylene after the coadministration of ethanol and cocaine, there is recent evidence that ethanol causes a significant increase in cocaine plasma concentrations when it is combined with cocaine (18). Thus, the presence of cocaine and ethanol not only can lead to the rapid formation of the more lethal cocaethylene but also may allow for a decrease in the catabolism of cocaine into its inactive metabolite benzoylecognine. With this dual effect

of decreased cocaine catabolism and increased cocaethylene production, higher levels of both the parent drug and the more toxic metabolite may occur and lead to the observed increase in mortality.

Cocaethylene has been detected at autopsy in the urine, blood, brain, and liver of individuals who were known to succumb to overdose while on both agents (4,20,23). Although this evidence indicates the formation of cocaethylene and suggests its contribution to the resultant toxicity, the mechanisms by which cocaethylene, as well as cocaine, produce lethality are currently unknown. Regardless of the underlying etiology of the apparent enhanced lethality produced by concurrent use of alcohol and cocaine, research directed toward investigating drugs that may reduce the toxic effects and thereby lower the instances of lethality for this commonly abused drug combination is needed. Because the exact cause of lethality produced by the combination is currently unknown, any agent capable of combating the toxic lethal effects will be extremely useful.

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