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Cocaine Toxicity: Genetic Differences in Cocaine-Induced Lethality in Rats

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GEORGE, F. R. *Cocaine toxicity: Genetic differences in cocaine-induced lethality in rats.* PHARMACOL BIOCHEM BEHAV **38**(4) 893–895, 1991.—Cocaine produces stimulation, depression, convulsions and death, and binds at several receptor sites. Thus cocaine may produce toxicity through similar or distinct pathways from those associated with stimulation. Genetic differences in stimulant response to cocaine have recently been reported for four inbred rat strains, ACI, F344, LEW and NBR. In the present study, significant genetic differences were found in cocaine-induced lethality, with a two-fold difference in LD_{50} values seen between the most sensitive (NBR) and least sensitive (LEW) strains. Sensitivity to lethality was not correlated with sensitivity to stimulation, but was highly correlated with baseline activity. This lack of strong association between stimulant and lethal effects of cocaine may be important in clinical manifestations of the cocaine sudden death syndrome.

Toxicity Lethality Cocaine Rats Behavior genetics

COCAINE, when administered systemically by any of several routes, produces numerous central and peripheral effects, primary among them being marked physiological responses (e.g., changes in heart rate and blood pressure) as well as behavioral effects including euphoria and central nervous system (CNS) stimulation (12,13). However, large doses of cocaine can induce stereotypy, disruption of schedule-controlled behavior, and severe toxicity, including seizures and death (5–7, 21). Unfortunately, with the recent increase in self-administration of crack, a highly potent and rapidly absorbed form of cocaine, the incidence of toxic responses to cocaine, especially seizures and death, has risen significantly (11).

While the number of reports is limited, results from existing studies suggest that genotype is a significant factor in determining the magnitude of behavioral responses to cocaine. Shuster et al. (19) showed that C57BL/6J mice were substantially more activated than A/J mice by 20 mg/kg cocaine. Ruth et al. (16) recently showed significant genetic differences in Y-maze activity, rearing activity and heart rate following cocaine. George (3) has shown that cocaine is more potent in producing low dose depressant effects in C57BL/6J relative to DBA/2J mice, while George and Ritz (7) and de Fiebre et al. (2) have shown that LS and SS mice differ substantially in their locomotor stimulant responses to cocaine. In addition, LS and SS mice have been recently shown to differ substantially in cocaine-induced seizures (2,4) but not lethality (4), suggesting that these two toxic responses to cocaine are mediated via distinct pathways.

It has recently been reported that large genetic differences exist in the locomotor stimulant effects of cocaine and amphetamine in rats from the ACI, F344, LEW and NBR inbred strains (6). However, significant strain by drug interactions were found, in that the strain rank order for stimulant response to the two drugs was not identical. Locomotor stimulant response to either of these two drugs also was not highly correlated with baseline levels of activity. In this same study, ligand affinity for and receptor density of dopamine transporters and dopaminergic D_1 and D_2 receptors in striatal tissue from these same strains of rats were also assessed. No differences in these receptor binding parameters were found. These findings support the conclusion that cocaine and amphetamine produce their locomotor stimulant effects through different sites of action, but that genetic differences in response to these drugs at the behavioral level do not appear to be mediated significantly by a major gene effect regulating structure or number of dopaminergic sites.

Since cocaine exerts a number of effects, and binds with micromolar affinity to several CNS sites, one approach to unraveling the mechanisms associated with the multiple effects of cocaine is the use of pharmacogenetic designs which compare relative responses to different drug effects across several defined genotypes. Thus the primary purpose of this study was to examine whether genetic differences exist in sensitivity to the lethal effects of cocaine in inbred rats whose sensitivity to the stimulant effects of cocaine has been established. A second purpose was to then compare the relationship between baseline ambulatory activity, locomotor stimulant, and lethal responses to cocaine across these rat strains, to provide initial data concerning the degree of genetic relatedness of these phenotypes.

METHOD

Animals

Adult (10–14 weeks) ACI/HSD (ACI), NBR/Nih (NBR), LEW/CRIBR (LEW), and CDF(F-344)/CRIBR (F344) male rats were used. All animals were bred and housed in the NIDA Addiction Research Center animal colony, and were first generation off-

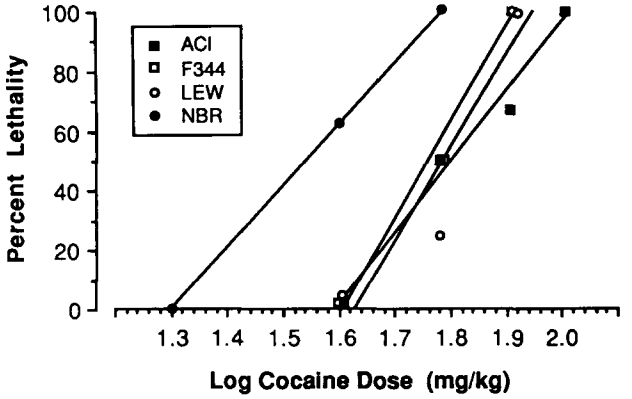

FIG. 1. Percent lethality as a function of the logarithm of cocaine dose in ACI, F344, LEW and NBR male rats. Log 20 mg/kg = 1.3, log 40 mg/kg = 1.6, log 60 mg/kg = 1.78, log 80 mg/kg = 1.9, log 100 mg/kg = 2.0.

spring of commercially purchased breeders. The animals used in this study were maintained in facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC) and the studies were conducted in accordance with the Guide for Care and Use of Laboratory Animals provided by the NIH. All animals were experimentally naive, and housed in groups of same sex littermates with ad lib access to Purina chow and tap water. All rats were maintained in a temperature controlled room (26°C) with a 12-h light-dark cycle (0700–1900 lights on).

Procedure

The rats were divided into treatment groups of N = 6–12 per group. Littermates were distributed across conditions. Rats were injected IP with one of the following doses of l-cocaine-HCl expressed as free base in 0.9% sterile saline: 0 (vehicle), 20, 40, 60, 80 or 100 mg/kg. All cocaine doses were administered in a volume of 1.0 ml/kg. All rats were tested between 900 and 1300 h. The rats were weighed, injected, and then individually placed in standard plastic rat cages without bedding. Rats were monitored for a period of 60 minutes postinjection. Occurrence of death during this period was recorded. All testing was conducted in an isolated room under reduced light.

Data Analysis

All analyses were conducted using SYSTAT for Macintosh (23). LD_{50} values were calculated using standard linear regression analyses of dose response curves for each genotype. Genotype comparisons were performed using multiple regression analyses for binary dependent measures (PROBIT) (22).

RESULTS

Cocaine produced dose-dependent lethality in all four strains, $t(\text{Dose})(96) = 5.65$, $p<0.0001$, as shown in Fig. 1. This figure also shows that significant genetic differences in sensitivity to the lethal effects of cocaine was seen, $t(\text{Strain})(96) = 2.06$, $p<0.02$. Rats from the NBR strain, with a LD_{50} value of 34.0 mg/kg, were significantly more sensitive to cocaine-induced lethality than animals from any of the other strains, showing a nearly two-fold greater sensitivity to this effect relative to ACI rats, which had a LD_{50} value of 64.3 mg/kg. LEW and F344 rats had LD_{50} values of 61.4 and 57.5 mg/kg respectively, and while they differed from the NBR rats, they were not different from the ACI rats in terms of cocaine-induced lethality. Table 1 lists the LD_{50} and ED_{50} for locomotor stimulation and baseline activity values for these rat strains.

TABLE 1

LOCOMOTOR ACTIVITY BASELINE SCORES, ED_{50} VALUES (mg/kg) AND LD_{50} VALUES (mg/kg) FOR ACI, F344, LEW AND NBR MALE RATS

Strain	Baseline Activity	Cocaine ED_{50}	Cocaine LD_{50}
ACI	225	12.4	64.3
F344	394	18.9	57.5
LEW	282	9.7	61.4
NBR	1123	6.4	34.0

Table 2 shows the correlations between sensitivity to the lethal effects of cocaine, sensitivity to the locomotor stimulant effects of this drug, and baseline ambulatory activity. As shown previously (6), locomotor stimulant response to cocaine is modestly but not significantly correlated with baseline levels of ambulatory activity. The present results show that sensitivity to cocaine-induced lethality is not significantly related to the locomotor stimulant effects of this drug ($p = 0.43$) (Table 2). However, as opposed to the locomotor stimulant effects of this drug, sensitivity to cocaine-induced lethality appears to be highly correlated with baseline levels of ambulatory activity ($p<0.01$).

DISCUSSION

The results of this study substantiate previous findings that cocaine produces lethality. Novel findings were obtained in that large genetic differences were seen in this response, and this response was not significantly related to the locomotor stimulant effects of cocaine. These results are consistent with other recent findings. For example, SS mice show a much greater locomotor stimulant response to cocaine relative to their LS counterparts (2,4), but do not differ in cocaine-induced lethality (4). Thus it appears that cocaine-induced locomotor activation and lethality are not dose-related aspects of a single behavioral and biological continuum, but are instead distinct responses mediated by different neuronal mechanisms or pathways. However, sensitivity to the lethal effects of cocaine does appear to be highly related to baseline levels of ambulatory activity, suggesting that these two phenotypes may have at least some common pathways. While the

TABLE 2

CORRELATIONAL RELATIONSHIPS BETWEEN LD_{50} VALUES FOR COCAINE-INDUCED LETHALITY, ED_{50} VALUES FOR COCAINE-INDUCED LOCOMOTOR STIMULATION AND BASELINE AMBULATORY ACTIVITY SCORES FOR ACI, F344, LEW AND NBR MALE RATS

	LD_{50}	ED_{50}	Baseline
LD_{50}	—	0.56	0.99*
ED_{50}		—	0.50
Baseline			—

For all values, $df = 2$. *Denotes $p<0.01$. ED_{50} and baseline activity data from George et al. (6).

specific mechanisms, i.e., cardiac vs. central, of cocaine-induced lethality remain undetermined, the apparent relationship between baseline levels of activity and lethality suggests that at least some of the lethal effects of cocaine are due to central mechanisms. While these results should be accepted with caution due to the relatively small number of genotypes employed in the present study, the magnitude of the relationship is striking and warrants more detailed investigation.

Rates of cocaine metabolism were not obtained in this study. However, the large difference between the most and least sensitive strains, and the fact that most of the deaths seen in this study occurred within ten minutes postinjection, with the strain differences in lethality remaining consistent throughout the entire length of the test session, makes it unlikely that the genetic differences in lethal response to cocaine seen between strains are due solely to pharmacokinetic factors, such as differences in hepatic metabolism or rates of conversion of cocaine to benzoylecgonine by plasma carboxyesterases. It is more likely that differences in neuronal, pharmacodynamic factors are primarily responsible for the differential sensitivities of these strains to the lethal effects of cocaine, although a pharmacokinetic contribution cannot be ruled out.

The CNS effects of cocaine are commonly thought to be due to the ability of cocaine to bind at sites associated with the three monoamine transporters, thus preventing the reuptake of dopamine, norepinephrine and serotonin (1, 9, 10, 14, 15, 20). At higher concentrations, cocaine has also been shown to bind to muscarinic (18) and sigma sites (17). Since cocaine acts at multiple sites and produces several effects, this makes elucidation of mechanisms of action associated with specific effects a relatively difficult task. Identifying differences in receptor populations or neurotransmitter levels among several rodent genotypes could aid in determining common and different sites of action for the several responses to cocaine.

The findings presented here raise several questions concerning effects of and responses to cocaine. One important question is whether genetic variation in lethal responses to cocaine such as that shown in the present study can be utilized to determine the mode of transmission and molecular basis of this trait. Answers to this and other related questions will contribute to our understanding of the specific nature of cocaine's actions as well as to the more general phenomenon of substance abuse.

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