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Behavior and Drug Measurements in Long–Evans and Sprague–Dawley Rats After Ethanol–Cocaine Exposure

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HOROWITZ, J. M., E. BHATTI, B. G. DEVI AND G. TORRES. *Behavior and drug measurements in Long–Evans and Sprague–Dawley rats after ethanol–cocaine exposure.* PHARMACOL BIOCHEM BEHAV **62**(2) 329–337, 1999.—Long–Evans and Sprague–Dawley rats show differential behavioral responses to cocaethylene, a metabolite derived from the simultaneous ingestion of ethanol and cocaine. Such differences may also be manifested when these outbred strains are exposed to ethanol and cocaine. To test this hypothesis, both strains were fed an ethanol-diet (8.7% v/v) in conjunction with cocaine (15 mg/kg) injections for 15 days. The following parameters were evaluated: (a) ethanol consumption, (b) cocaine-induced behavioral activity, (c) blood ethanol levels, (d) blood, liver, or brain cocaine and cocaethylene levels, and (e) liver catalase and esterase activity. We found that Long–Evans rats drank significantly more of the ethanol diet relative to the Sprague–Dawley line during the first few days of the test session. This rat phenotype also differed significantly from the Sprague–Dawley line in terms of behavioral activity after cocaine administration. Blood ethanol levels did not differ between strains. Similarly, we failed to detect strain-dependent differences in blood, liver, or brain cocaine levels as measured by gas chromatography/mass spectrometry. Cocaethylene levels, however, were higher in blood and brain of Long–Evans relative to Sprague–Dawley cohorts. Although the ethanol–cocaine regimen produced a marked suppression of catalase and esterase activity compared with control-fed rats, this suppression was roughly equivalent in both rat phenotypes. These data are discussed in the context of genotypic background and vulnerability to polysubstance abuse. © 1999 Elsevier Science Inc.

Cocaethylene Genotype Blood Liver Striatum Catalase Esterase

RECENT epidemiological studies indicate that the simultaneous use of ethanol and cocaine is a common pattern of drug consumption in humans (18). Despite its prevalence, very little research has been aimed at identifying the possible underlying biological substrates mediating the combined use of highly addictive drugs. It is conceivable that cocaethylene, a metabolite derived from the in vivo transesterification of cocaine by ethanol-dependent enzymes, may contribute significantly to this pattern of substance abuse by acting at specific neural substrates linked to drug use and drug craving (35,40). Indeed, cocaethylene is likely to have psychotropic effects in common with cocaine because it (a) has a high affinity for the dopamine (DA) transporter site (21), and (b) acts as a powerful reinforcer in self-administration and drug-discrimination studies (26,38). All of these effects appear to take place in discrete neurons of the mesoaccumbens projection, a neural circuit strongly implicated in drug-induced reward (29). Therefore, cocaethylene may have reinforcing properties of its own, thereby accentuating the binge use of ethanol and cocaine in humans.

Although the actions of cocaethylene undoubtedly account for some aspects of ethanol and cocaine consumption, it is also likely that genetic factors could explain the basis for vulnerability to polysubstance abuse behavior, especially as they relate to interindividual differences. In this context, a variety of rodent and human data suggest that genetic background is linked to individual differences in vulnerability to develop substance abuse (6,15,17). For instance, Lewis and Fischer-

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344 rats have been used as comparative mammalian models to examine the neural correlates of addictive behavior because they show marked differences in their behavioral and neurochemical profile to consume ethanol and other psychoactive drugs (14,19,20,31,32). The recognition that specific strains of rats display contrasting responses to ethanol and cocaine is of considerable interest because they can be used to characterize genotype-dependent differences in sensitivity to drug exposure. Despite extensive investigation, however, the biological basis for most of the behavioral or neurochemical differences between rat strains is unknown. Because both ethanol and cocaine share the ability to increase the concentration of DA and serotonin [5-hydroxytryptamine (5-HT)] in receptive terminal fields of the mesoaccumbens region, strain differences in the behavioral responses to these drugs may be related to differences in DA or 5-HT neurotransmission. Indeed, we have recently shown that synaptic 5-HT bioavailability may be an important element in mediating the behavioral differences to cocaethylene between Long–Evans and Sprague–Dawley rats (23,24). Because these outbred strains diverge significantly in their behavioral responses to cocaethylene, they may also be useful in the search for the endogenous mechanisms or correlates that underlie sensitivity to combined ethanol-cocaine administration. Such analyses can also favor the search for correlation(s) between genotype and a particular phenotypic trait, which can then be studied further by adopting molecular approaches in inbred rats. It should be noted however, that behavioral differences between outbred rat strains are multifactorial and, therefore, cannot be explained entirely by differences in specific neurochemical systems. Thus, although we inferred a relationship between behavioral activity and 5-HT bioavailability in Long–Evans and Sprague–Dawley rats (24), apparent changes in this neurotransmitter alone cannot adequately account for all the variation in responsiveness to cocaethylene (2). Other variables, such as drug metabolism and/or drug interactions, may be related to previously observed behavioral differences between Long–Evans and Sprague–Dawley rats.

To our knowledge, there are no published studies describing the combined behavioral effects of ethanol–cocaine in different rat strains. Moreover, very little is known about the relative concentrations of ethanol and cocaine in blood, liver, and brain following a chronic schedule of polydrug administration. This is of potential interest because it might provide an informative baseline from which to study possible ethanol–cocaine interactions, especially as they relate to behavior and drug biotransformation. In addition, an unexamined dimension is the impact exerted by ethanol and cocaine on specific enzymes that regulate their respective metabolic pathways. Although the importance of catalase and esterase activities in inactivating ethanol and cocaine is well known, pharmacokinetic changes in these two liver enzymes after combined ethanol-cocaine exposure have not been reported. In the case of ethanol, catalase oxidizes it into acetaldehyde, whereas (carboxyl) esterase hydrolyzes cocaine into ecgonine methyl ester and benzoylecognine (9,25). Therefore, it may be important to evaluate the intrinsic effects of highly addictive drugs on catalase and esterase activities, especially as they relate to strain differences in drug sensitivity. Against this background, coupled with the findings that Long–Evans and Sprague–Dawley rats show substantial behavioral differences to cocaethylene, we hypothesized that there might also be significant differences between the aforementioned strains in behavioral activity during chronic, combined ethanol–cocaine administration. Furthermore, because these drugs are frequently used in combination, the present studies were designed to measure levels of ethanol and cocaine in various central and peripheral tissues. Moreover, because this drug combination results in the formation of cocaethylene, we also measured the levels of this ethyl metabolite in liver and brain of Long–Evans and Sprague–Dawley rats. The brain region selected for cocaethylene analysis was the striatum (caudate putamen and nucleus accumbens), a telencephalic region that serves as a common substrate for the behavioral and reinforcing actions of drugs (30). Finally, we determined hepatic levels of catalase and esterase activity after chronic ethanol and cocaine administration in both rat strains.

METHOD

Male Long–Evans and Sprague–Dawley rats, weighing approximately 220 g upon arrival, were purchased from Harlan Laboratories (Indianapolis, IN) and housed at the University vivarium. The rats were placed in groups of three per cage with constant temperature (22°C) and humidity (60%) and maintained on a 12 L: 12 D cycle with lights off at 1700 h. For 5 days prior to testing, all animals were fed Purina lab chow and water ad lib and handled daily to minimize the possibility of nonspecific stress. On the first day of the study, the rats were housed individually and fed a liquid ethanol diet (see below) for 15 days. It should be noted that because detection of cocaethylene was a significant aim in the present studies, control groups were not instituted in experiments measuring the metabolite. The reason for this is that cocaethylene synthesis occurs only in the presence of ethanol and cocaine (3). All testing procedures were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and with the approval from the State University of New York at Buffalo IACUC.

Ethanol Liquid-Diet Procedure

The ethanol liquid diet used in these studies has been described in detail previously (11), and is only briefly summarized here. The ethanol liquid diet was prepared daily and consisted of ethanol (8.7% v/v) supplemented with vitamins (ICN, OH; 0.31% w/v), minerals (ICN, OH; 0.50%), and chocolate sustacal (83.3% v/v). This liquid ethanol diet was chosen because (a) it is a well-established method of ethanol administration in rodents, and (b) this mode of administration approximates the method of consumption in humans. The amount of ethanol content was introduced to the rats gradually, to increase the likelihood of acceptance by adding one-third of the total ethanol on the first day, two-thirds on the second day, and the full ethanol content on the third day. Administration of the full ethanol content was considered to be day 1 of the study. The liquid ethanol diet was fed to the rats in graduated drinking bottles placed in the home cages. Daily consumption was recorded at 1700 h prior to refilling the bottles with fresh ethanol solution. To ensure proper weight gain in all rats throughout the course of the study, body weights were recorded on days 5, 10, and 15.

Cocaine Administration

Long–Evans and Sprague–Dawley rats were injected intraperitoneally (IP) with cocaine hydrochloride (Sigma; St. Louis, MO) dissolved in 0.9% saline at a dose of 15 mg/kg (calculated as the free base). Starting on day 1, the rats were injected daily for 15 days at 1900 h. Given that rats consume most of the liquid ethanol diets during the dark phase of the

LD cycle, it was decided that injections at this particular time would ensure that high cocaine levels coincided with increased ethanol content in blood.

Behavior Analysis

Following daily cocaine injections, the rats were observed (under a dim red light) in their respective home cages for behavioral activity by an individual "blind" to the experimental procedures. Using a 10-point rating scale (12,28), behavioral activity on days 1, 5, and 10 was rated 5 min prior to the cocaine injection and every 5 min thereafter for 30 min. This particular behavioral rating scale was chosen because it provides an excellent estimate of behavioral activity ranging from exploratory behavior to stereotypy (24), and also because it allows for comparison across behaviorally based studies. At every observation, a score was recorded on a scale from 1–10, where: 1 = asleep or still; 2 = inactive or in-place activity; 3 = locomotion (all 4 feet moving within a 10-s period), rearing, or sniffing (>3 s duration); 4 = any combination of two of locomotion, rearing, or sniffing; 5 = continuous sniffing for 10 s without locomotion or rearing; 6 = continuous sniffing for 10 s with locomotion or rearing; 7 = patterned sniffing for 5 s; 8 = patterned sniffing for 10 s; 9 = continuous gnawing; 10 = bizarre diskinetic movements or seizures.

It is important to note at the dose of cocaine administered, we never observed scores greater than 6 in the present set of experiments.

Experimental Procedures

One of the aims of this study was to measure ethanol levels in blood of Long–Evans and Sprague–Dawley rats at various times during chronic ethanol–cocaine administration. To this end, tail blood was collected from each subject on day 5 of the study at 1900 h. Briefly, animals were removed from their home cages and approximately 20 μl of blood was collected from a cut made at the tip of the tail. The blood was drawn into ice-cold heparinized capillary tubes, centrifuged at 10,000 rpm for 10 min, and stored frozen at −70°C until assayed for ethanol content. For the measurement of blood ethanol levels on day 15, trunk blood was collected at 1900 h in ice-cold microtubes containing 60 μl EDTA (50 mg/ml). All subsequent procedures were identical to those described for tail blood collection. It should be noted that trunk blood appears to be a more accurate measure of ethanol consumption than blood derived from tail vein (34). Therefore it is possible that ethanol levels reported on day 5 in the present study may have been underestimated.

Blood Ethanol Analysis

The simultaneous measurement of ethanol content in tail and trunk blood collected from Long–Evans and Sprague–Dawley rats on days 5 and 15, respectively, was performed using a commercially available NAD+ alcohol dehydrogenase Assay Kit (Sigma), which allows for a quick, accurate measurement of blood ethanol levels using spectrophotometric methods. Briefly, 3 ml of glycine buffer were added to 10 μl of plasma or blanks followed by additional 3 ml of a NAD–ADH solution. All samples were then incubated for 10 min at room temperature. One milliliter of each sample was pipetted into cuvettes and absorbance read at a wavelength of 340 nm against blanks. Readings were made in a Beckman spectrophotometer. Blood ethanol levels are expressed as mg/dl.

Blood, Liver, and Brain Cocaine Analysis

Measurement of cocaine levels has considerable relevance to this study because differences in pharmacokinetics may contribute to strain-dependent differences in behavior. In addition, concentrations of cocaine achieved during chronic ethanol–cocaine administration have not been reported, especially from different rat strains. Because cocaine has a short half-life and a relatively complete systemic metabolism after its administration (25), we collected blood, liver, and brain samples from Long–Evans and Sprague–Dawley rats immediately after the last cocaine injection. Briefly, on the last day of testing (i.e., day 15) and 15 min after the last cocaine injection, all rats were anesthetized with CO_2 and then decapitated between 1900 and 1930 h in a room adjacent to the animal vivarium. Trunk blood was collected as described previously, with the exception that microtubes coated with EDTA also contained 4% NaF. The inclusion of NaF was done to prevent cocaine hydrolysis by nonspecific plasma cholinesterases (3). Liver samples were collected from the anterior right lobe by excising only a discrete portion of the liver (~20 mg/sample) and placing it directly into ice-cold microtubes containing 1 ml of high-grade HPLC water. Each individual liver sample was homogenized, centrifuged at 25,000 rpm for 20 min, and the supernatant (200 μl/sample) treated with 10 μl 4% NaF. Brains were rapidly removed from the calvaria and microdissected (minus the cortices) into the caudate putamen and nucleus accumbens (two major components of the striatum). The extent of the microdissected brain tissue ranged from bregma 1.70 mm (plate 11) to bregma −0.40 mm (plate 21) of the rat atlas of Paxinos and Watson (36). Brain sections were homogenized in 2 ml of high-grade HPCL water, centrifuged at 25,000 rpm for 20 min, and the supernatant (1 ml/sample) also treated with 4% NaF. Blood, liver, and brain samples were then immediately frozen on dry ice and stored at −70°C until the simultaneous determination of cocaine and cocaethylene levels by capillary gas chromatography/mass spectrometry under the supervision of Dr. David M. Andrenyak at the University of Utah (see below).

Blood, Liver, and Brain Cocaethylene Analysis

We measured cocaethylene content in blood, liver, and brain of Long–Evans and Sprague–Dawley rats exposed to ethanol and cocaine for 15 consecutive days. The collection procedure(s) of the above samples were identical to those described previously. The extraction and subsequent measurement of cocaine and cocaethylene were modifications of the methods reported by Crouch et al. (8). Briefly, blood, liver, and brain samples were treated with 50 ng cocaine and 50 ng cocaethylene (deuterated internal standards) and 4 ml of 100 mM sodium acetate buffer; the samples were then centrifuged at 1000 × *g* for 5 min. Using solid phase extraction, cocaine and cocaethylene were extracted from fluid and tissue samples and loaded into columns treated with 3 ml of methanol:2 ml water:2 ml of 100 mM sodium acetate buffer. After sample loading, the columns were washed with a mixture of 2 ml of 100 mM HCl:3 ml of methanol solution. The columns were then dried for 5 min under vacuum and eluted with 3 ml methanol and concentrated ammonium hydroxide. The extracts were collected in glass culture tubes, evaporated to dryness and derivatized with 100 μl hexafluoroisopropanol and 100 μl pentafluoropropionic anhydride. The residues were heated at 80°C for 45 min, cooled to room temperature, evaporated to dryness, and reconstituted with 50 μl ethyl acetate. The derivative extracts were then analyzed by capillary gas chromatog-

raphy/mass spectrometry. Measurements of cocaine and cocaethylene concentrations in the samples were extrapolated using a standard curve generated from blood samples spiked with known amounts of cocaine and cocaethylene. Based on a 1-ml sample, the limit of quantification for both cocaine and cocaethylene was 1 ng/ml.

Analysis of Catalase and Esterase Activity in Hepatocytes

Liver samples were collected from both strains as described above. To compare changes in enzymatic activity in rats exposed to 15 days of ethanol and cocaine with those maintained on tap water and food pellets alone, a subset of male Long–Evans and Sprague–Dawley control rats (n = 3/group) were decapitated (after CO_2 anesthesia) at 1900 h and their livers excised and stored at −70°C until determination of catalase and (carboxyl) esterase activities. On the day of the assays, liver samples were homogenized in ice-cold 0.1 PO_4 buffer (pH: 7.2) using a glass mortar and Teflon pestle. The homogenates were centrifuged (1000 × g for 10 min) to remove protein and other cellular debris, and the pH of the acid soluble supernatant was adjusted to 7.0 before each appropriate assay. The supernatants were taken for protein estimation and enzymatic assays according to the procedure described for catalase and esterase activity (1,27). The standards and samples were extracted with 1-butanol before they were scanned between 500 to 550 nm on a Beckman Spectrophotometer. Protein estimation was done by the bicinchoninic acid method (39), using bovine serum albumin as a standard. Liver catalase activity is expressed as units/mg/protein, whereas liver esterase activity is expressed as mol × 10^{-6}/min/g wt tissue.

Data Analysis

Statistical approaches directed at the analysis of behavioral activity, drug levels, and liver enzymatic activity in Long–Evans and Sprague–Dawley rats exposed to ethanol and cocaine involved a two-way ANOVA and, if significant ($p \leq 0.05$), subsequent post hoc Student's t-test. For analysis of differences to the stimulatory effects of cocaine, a two-way ANOVA with independent measures was used to test for the effect of strain at selected time points. For the pharmacological data, rats were grouped by strain and drug–tissue measurements. Overall differences among drug levels were assessed by unpaired two-tailed Student's t-tests. Statistical analysis for enzyme activity between treatment and control values used a two-way ANOVA (treatment × strain) with repeated measures on one factor. Student's t-tests were used to determine specific mean comparisons between rat lines. Data are expressed as means ± SEM.

RESULTS

Ethanol Consumption and Body Weight Parameters

Daily consumption of the ethanol liquid diet was recorded for both rat strains. As depicted in Fig. 1, Long–Evans ingested significantly ($p \leq 0.05$) more ethanol during the first 3 days of the drug regimen than did Sprague–Dawley rats. This difference, however, was no longer apparent after the fifth day of ethanol consumption. It should be noted that although not statistically significant, Long–Evans rats continued to drink more than the Sprague–Dawley strain throughout the remaining days of the ethanol treatment (see Fig. 1). To examine the possibility that there might be an innate quantitative difference in the drinking patterns of Long–Evans and Sprague–Dawley rats, a second (corroborative) experiment was conducted to determine the relative levels of tap water consumed by each rat line over a period of 5 days. Water consumption for Long–Evans rats (n = 5) on day 5 was (means ± SEM) 24 ± 2.9 ml, whereas for Sprague–Dawley rats (n = 5) it was 27 ± 2.2 ml ($p \geq 0.05$ between strains with no significant interaction between strains and time). Therefore, the higher levels of ethanol ingested by Long–Evans rats are not explained by innate preferences in drinking per se. It is noteworthy that no visible signs of ethanol-induced ataxia were observed in either strain at any time throughout the 15 days of ethanol consumption. In general, our results show that Long–Evans drink more ethanol that Sprague–Dawley rats. This strain difference in fluid ingestion, however, is transient and appears relatively specific to ethanol.

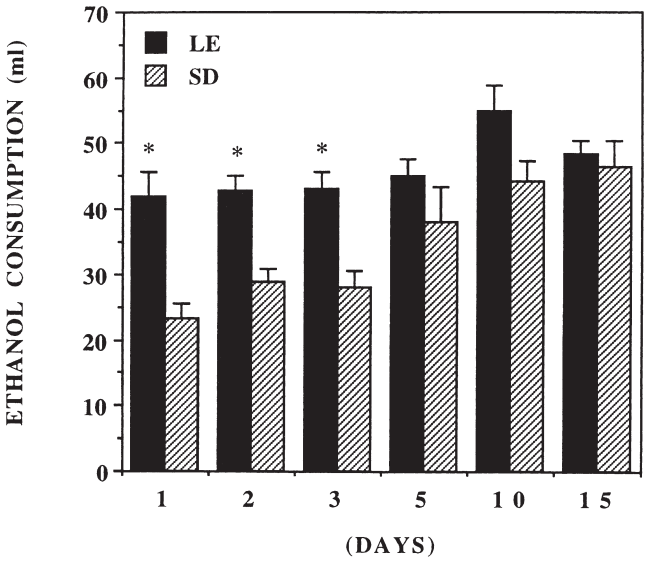

FIG. 1. Ethanol consumption in Long–Evans (LE) and Sprague–Dawley (SD) rats on depicted days of the combined ethanol–cocaine regimen. The incidence of ethanol consumption was greater (*$p \leq 0.05$) in LE relative to SD rats. It should be noted that the observed divergence in drinking patterns was prominent only in the first 3 days of drug exposure. Ethanol consumption values after this time did not differ significantly between strains, as shown on days 5, 10, and 15 of the study. Graph bars represent the mean ± SEM values of ethanol consumed (in ml) by each rat phenotype. Two-way repeated-measures ANOVA on two factors showed a significant main effect of strain, $F(1, 29) = 17.61$, $p \leq 0.005$, day, $F(5, 29) = 19.6$, $p \leq 0.0001$, and strain × day interaction, $F(5, 29) = 4.30$, $p \leq 0.004$.

Body weights recorded on day 5 of the ethanol treatment revealed that Sprague–Dawley rats lost about 5% of their original body weight (means ± SEM) 210.1 ± 1.7 g on day 1, 199.0 ± 3.6 g on day 5. This may be explained, however, by the fact that this strain line was briefly adipsic to the ethanol liquid diet. From day 5 on, a gradual increase in body weight was observed for all Sprague–Dawley rats. For instance, on day 15, body weights recorded were 225 ± 5.3 g. This represents a nearly 13% increase in body weight over a 10-day period. In contrast, mean body weights of the ethanol-treated Long–Evans rats were not significantly affected during the first 5 days of drug treatment. For example, on day 5, body weights recorded were 231 ± 3.6 g, whereas on day 15 they were 263.8 ± 8.2 g. This weight gain, similar to that observed

in the Sprague–Dawley line, represents a 14% increase in body weight over a 10-day period. Therefore, Long–Evans and Sprague–Dawley rats did not differ significantly in body weight gain, especially during the last 10 days of ethanol consumption. This, along with the fact that these rat lines ingested equivalent amounts of lab chow prior to testing (data not shown), suggests that the observed high levels of ethanol intake in Long–Evans rats are not calorically driven.

Behavioral Effects of Combined Ethanol–Cocaine Exposure

To examine the stimulatory effects of cocaine in rats treated simultaneously with ethanol, we measured the behavioral activity of Long–Evans and Sprague–Dawley rats on days 1, 5, and 10. As expected, all rats injected with 15 mg/kg cocaine on day 1 of the combined drug treatment exhibited increased behavioral activity. At this dose, cocaine HCl elicited strong bouts of continuous sniffing with locomotion and rearing in both strains. For instance, Long–Evans showed behavioral ratings of (means ± SEM) 5.7 ± 0.2, whereas Sprague–Dawley rats showed ratings of 5.42 ± 0.4 ($p \geq 0.05$). Interestingly, on days 5 and 10, the strong behavioral response to cocaine was significantly ($p \leq 0.05$) attenuated in Long–Evans but not Sprague–Dawley rats (Fig. 2). It should be noted that decreases of behavioral activity to cocaine in the Long–Evans line was not observed in all rats tested, as two of these rats displayed robust episodes of sniffing and rearing. Regardless of this, and despite the fact that both strains ingested the same 8.7% ethanol solution, Long–Evans showed blunted behavioral responses to cocaine relative to those observed in Sprague–Dawley rats. Furthermore, this difference in behavioral activity levels between rat lines lasted until the end of the ethanol–cocaine treatment (data not shown).

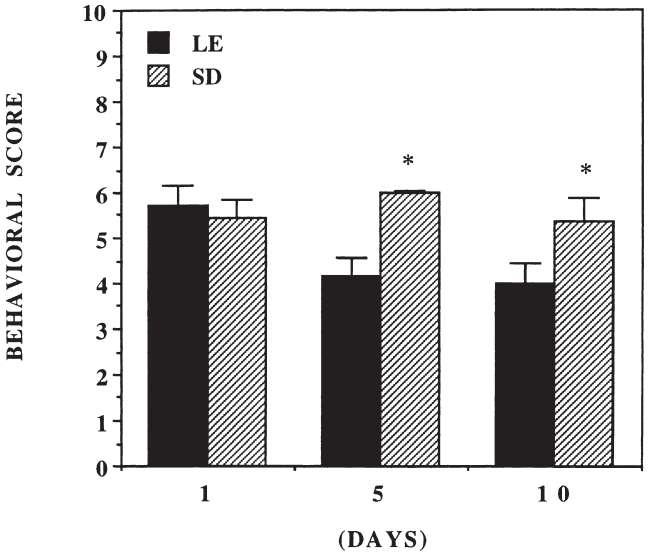

FIG. 2. Behavioral activity in ethanol-fed Long–Evans (LE) and Sprague–Dawley (SD) rats after daily cocaine (15 mg/kg) injections on depicted days of drug treatment. Note that the incidence of behavioral activity is significantly (*$p \leq 0.05$) blunted in LE relative to SD rats on days 5, $t(12) = 2.71$, $p \leq 0.01$, and 10, $t(12) = 2.2$, $p \leq 0.04$, of the study. Such a behavioral difference is not observed during the first 3 days of the combined ethanol–cocaine regimen. Graph bars represent the mean ± SEM of behavioral rating scores for each rat phenotype.

Blood Ethanol Levels

While there were inter- and intraindividual differences, both strains consumed sufficient amounts of the diet to reach blood ethanol concentrations in the range of 18–160 mg/dl. These drug levels are within the range of those described by us (41), and are within levels of intoxication in humans. Ethanol measurements were made from tail vein and trunk blood collected on days 5 and 15, respectively. Concentrations of this drug in Long–Evans tail blood were (means ± SEM) 48.1 ± 19.3 mg/dl, whereas blood concentrations for Sprague–Dawley rats were 69.0 ± 16.9 mg/dl. These ethanol levels did not markedly differ ($p \geq 0.05$) between the rat lines. Similarly, trunk blood levels on day 15 did not vary significantly ($p \geq 0.05$) between Long–Evans and Sprague–Dawley rats. For instance, blood ethanol levels measured in Long–Evans were 121.48 ± 18.85 mg/dl compared with 94.1 ± 10.5 mg/dl in Sprague–Dawley rats.

Blood, Liver, and Brain Cocaine Levels

Table 1 presents cocaine concentrations obtained on day 15 from Long–Evans and Sprague–Dawley rats. Fifteen minutes postinjection, blood cocaine levels were in the range of (means ± SEM) 504 ± 73 ng and 725 ± 79 ng. Significant intra- and interanimal variability was noted. Comparison between strains revealed no marked differences ($p \geq 0.05$) in terms of cocaine recovery from blood fluid or liver tissue. In addition, no significant differences ($p \geq 0.05$) in levels of cocaine measured in the striatum were detected between the rat

TABLE 1

CAPILLARY GAS CHROMATOGRAPHY/ MASS-SPECTROMETRY MEASURES OF BLOOD, LIVER, AND BRAIN COCAINE CONCENTRATIONS OF LONG–EVANS AND SPRAGUE–DAWLEY RATS AFTER 15 DAYS OF ETHANOL AND COCAINE ADMINISTRATION.*

	Cocaine (ng/ml)		
	Blood	Liver	Striatum
Long–Evans	840.1	196.5	693.8
	654.6	80.8	527.7
	1033.1	1157.1	1696.9
	550.4	203.5	478.5
	757.0	479.4	949.2
	518.5	189.0	675.5
	266.3	83.9	324.3
	660.6 ± 93.7	341.4 ± 145.2	763.7 ± 172.7
Sprague–Dawley	693.3	596.5	940.0
	416.2	145.2	427.3
	385.8	244.6	353.0
	657.6	187.7	525.1
	677.2	624.7	791.9
	529.6	73.3	447.7
	169.3	128.0	70.3
	504.1 ± 73.1	285.7 ± 86.4	507.9 ± 108.6

*No significant differences ($p \geq 0.05$) were found in blood, liver, or brain cocaine concentrations between Long–Evans and Sprague–Dawley rats. It should be noted that there is more variability in blood (plasma) levels when cocaine is administered intraperitoneally than intravenously (35). Therefore, the variability from our data most likely reflects mode of cocaine delivery. Tabulated data are means ± SEM.

lines. In general, our results show that cocaine concentrations in blood, liver, and brain are not different between Long–Evans and Sprague–Dawley rats.

Blood, Liver, and Brain Cocaethylene Levels

Liver cells contain carboxylesterases responsible for the catalysis of cocaine to cocaethylene in the presence of ethanol (10). This pharmacologically active metabolite is rapidly formed and detected in fluids and body tissues. In the present experiment, all rats administered with ethanol and cocaine exhibited measurable amounts of cocaethylene. The concentration of cocaethylene in blood, liver, and striatum represented approximately 9, 8, and 10% of the cocaine concentration, respectively. Similar findings in terms of greater concentrations of cocaine relative to those of cocaethylene have been reported in the literature (3,41). Statistical analyses revealed significant differences ($p \leq 0.05$) in blood cocaethylene levels between Long–Evans and Sprague–Dawley rats (Table 2). Long–Evans exhibited almost twice as much cocaethylene content in blood as did Sprague–Dawley rats. This strain difference also extended to brain but not liver cocaethylene levels.

Liver Enzymes Analysis

Under basal, nondrug conditions, activity levels for catalase and esterase were approximately 300 units/mg protein wet tissue and 1.1 mol $\times$ 10^{-6}/min/g, respectively (Table 3). These values did not differ ($p \geq 0.05$) markedly between Long–Evans and Sprague–Dawley rats. Statistical analyses showed that chronic ethanol–cocaine administration did not result in strain differences ($p \geq 0.05$) in either catalase or esterase activity. However, the activity of both enzymes was significantly reduced ($p \leq 0.05$) in drug-treated rats when compared with control animals. This enzymatic reduction was of approximately equal magnitude for both rat lines (Table 3).

DISCUSSION

The present study was undertaken to examine the possibility that differences in drug levels may account for the behavioral differences observed between Long–Evans and Sprague–Dawley rats. This has significant clinical value because many human cocaine users also ingest ethanol to enhance euphoria phenomena and/or to reduce the acute dysphoric symptoms that often accompany the cessation of a cocaine binge episode (18). Furthermore, addictive behavior in humans shows considerable individual variation. Therefore, genotype-based variants may contribute significantly to interindividual differences in vulnerability to alcoholism and cocaine abuse. The results of the present study indicate several innate differences between Long–Evans and Sprague–Dawley rats to moderate doses of ethanol and cocaine.

Both Long–Evans and Sprague–Dawley rats consumed modest amounts of ethanol. However, ethanol intake by Long–Evans exceeded that of Sprague–Dawley rats, especially during the first 3 days of ethanol availability. These results could reflect innate differences in ethanol preference, as genetic background is known to alter the sensitivity and/or tolerance to some ethanol effects (6). They could also reflect differences related to novelty and habituation to the liquid ethanol diet. It may be that Sprague–Dawley rats have a slower rate of habituation to the diet, and therefore, are extremely neophobic during the first few days of ethanol avail-

TABLE 2

CAPILLARY GAS CHROMATOGRAPHY/MASS SPECTROMETRY MEASURES OF BLOOD, LIVER, AND BRAIN COCAETHYLENE CONCENTRATIONS OF LONG–EVANS AND SPRAGUE–DAWLEY RATS AFTER 15 DAYS OF ETHANOL AND COCAINE ADMINISTRATION*

	Cocaethylene (ng/ml)		
	Blood	Liver	Striatum
Long–Evans	75.9	10.4	61.7
	91.3	7.1	85.0
	125.3	170.7	164.4
	45.2	34.2	61.3
	68.5	37.5	90.1
	69.1	14.0	92.4
	79.2 ± 11.0*	45.6 ± 25.6	92.4 ± 15.5*
Sprague–Dawley	67.4	24.0	109.6
	56.5	10.0	50.7
	10.2	18.7	9.2
	73.6	12.9	64.0
	46.0	50.3	56.4
	28.9	7.0	34.0
	9.9	15.1	13.9
	41.7 ± 9.8	19.7 ± 5.5	48.2 ± 12.9

*Significant differences ($p \leq 0.05$) in cocaethylene concentrations in blood and brain, but not liver, were found between Long–Evans and Sprague–Dawley rat stains. Note the high incidence of variability in cocaethylene levels in both rat lines. Tabulated data are means ± SEM. Statistical approaches directed at the analysis of cocaethylene content between strains in blood and striatum are as follows: $t(11) = 2.54$, $p \leq 0.02$, and $t(11) = 2.2$, $p \leq 0.04$, respectively.

TABLE 3

SPECTROPHOTOMETRIC MEASURES OF CATALASE AND ESTERASE ACTIVITY IN LIVER CELLS FROM LONG–EVANS AND SPRAGUE–DAWLEY RATS AFTER 15 DAYS OF LABORATORY DIET (CONTROL) OR COMBINED ETHANOL-COCAINE (DRUG) ADMINISTRATION*

	Catalase units/mg protein	Esterase mol χ 10^{-6}/min/g
Long–Evans		
Control	297.0 ± 22.0*	0.98 ± 0.11*
Drug	213.0 ±35.0	0.56 ± 0.09
Sprague–Dawley		
Control	312.0 ± 24.0*	1.12 ± 0.01*
Drug	244.0 ± 15.0	0.70 ± 0.01

*No strain-dependent differences ($p \geq 0.05$) in basal catalase or esterase activity were found between Long–Evans and Sprague–Dawley rats. However, activity levels of the aforementioned hepatic enzymes differed ($p \leq 0.05$) markedly between control and drug-treated rats within each strain. Long–Evans catalase activity, $t(5) = 3.19$, $p \leq 0.02$; esterase activity, $t(5) = 6.2$; $p \leq 0.001$. Sprague–Dawley catalase activity, $t(8) = 5.14$, $p \leq 0.001$; esterase activity, $t(8) = 26.9$, $p \leq 0.0001$. Data presented are means ± SEM.

ability. Changes in drinking patterns in response to a novel test environment could also explain the weight loss experienced by the aforementioned rat line. It is conceivable that reductions in body weight of Sprague–Dawley rats observed on day 5 can be attributed to stress produced by the shift from a solid diet to one that was entirely liquid derived. Long–Evans rats, in contrast, were relatively resistant to this dietary shift, therefore suggesting possible differences in the mechanisms mediating these effects. Previous studies have linked ethanol drinking and brain 5-HT function in both rats and humans. By and large, this linkage posits that ethanol-preferring animals have abnormally low 5-HT levels in some, but not all, brain regions (7). Because this neurotransmitter contributes significantly to the regulation of behaviors such as mood, arousal, and impulsivity (13), it is feasible that differences in ethanol consumption in Long–Evans and Sprague–Dawley rats could be ascribed to differences in brain 5-HT levels. Experiments addressing this issue are presently in progress.

Although there have been a vast number of reports depicting the stimulatory effects of cocaine alone, to our knowledge there have been no studies on the behavioral effects of combined ethanol–cocaine administration. We found that cocaine, under this drug regimen, produced a blunted behavioral response in Long–Evans but not Sprague–Dawley rats. In the latter strain, cocaine produced behavioral activity largely characterized by rearing, sniffing, and repetitive head and body movements. In contrast, Long–Evans rats showed minimal behavioral activity to 15 mg/kg cocaine. This difference was apparent by the second or third day of a cocaine injection to Long–Evans rats and was maintained steadily throughout the remaining days of testing. That Long–Evans show a lesser behavioral response to ethanol–cocaine administration than Sprague–Dawley rats suggests that there may be strain differences within neurochemical correlates of motor behavior. In this respect, there are reports of inbred strains showing differences in tyrosine hydroxylase (20), the rate-limiting enzyme for DA synthesis, and qualitative differences in the number of DA-containing neurons of the ventral tegmental area (VTA). These neurochemical and anatomical differences could contribute significantly to strain differences in sensitivity to ethanol or cocaine. Of interest, our laboratory has shown that Long–Evans are also less sensitive to the behavioral effects of cocaethylene when compared with Sprague–Dawley rats. However, this sensitity-based difference was not correlated to structural differences in neuronal density of DA or 5-HT-containing neurons of the VTA or raphé nucleus (24). Therefore, the basis for the differences in behavioral responsivity to cocaethylene in Long–Evans and Sprague–Dawley rats may involve a more global mechanism than just differences in the absolute numbers of nerve cells of the dopaminergic or serotonergic pathways. Regardless of the mechanism(s) underlying strain differences, the relative lack of behavioral impact of cocaethylene in Long–Evans rats also extends to the ethanol–cocaine regimen. Therefore, the Long–Evans strain shows an attenuated behavioral sensitivity to ethanol and cocaine when coingested together and also to their active metabolite, cocaethylene.

Although we found differences in ethanol intake between Long–Evans and Sprague–Dawley rats, blood ethanol levels did not differ between the two stains. The lack of significant differences on days 5 or 15 of drug exposure lead us to reject the possibility that differences in ethanol consumption have a pharmacokinetic basis. Ethanol levels in the present study ranged from 6 to 135 mg/dl in tail blood and from 65 to 156 mg/dl in trunk blood. These ethanol levels are in general agreement with our previous results (41), and with other ethanol studies as well (37). Therefore, these rat lines, with distinct drinking patterns, did not show pronounced differences in blood ethanol content. It should be noted that our reported ethanol–cocaine levels, when compared with those for ethanol alone, show no discernible differences in ethanol metabolism that could account for the strain differences in drinking behavior. Therefore, the reason(s) for the differences in ethanol intake between Long–Evans and Sprague–Dawley rats remains unknown and cannot be determined entirely from this study.

Significant increases in cocaine levels were observed in blood, liver, and striatum of Long–Evans and Sprague–Dawley rats after 15 days of ethanol–cocaine administration. However, the samples assayed for cocaine 15 min after the last cocaine injection did not differ between strains. This could be a reflection of the significant intra- and interanimal variability observed. In addition to cocaine, we also measured cocaethylene levels from the same described samples. Here we found significant differences in blood and striatal cocaethylene content, with Long–Evans showing greater concentrations of the ethyl metabolite than Sprague–Dawley rats. Interestingly, liver cocaethylene levels did not differ markedly between rat lines. Cocaine levels, irrespective of strain, were invariably higher than those for cocaethylene in all of the samples examined. Consistent with the present study, several investigators have demonstrated similar blood cocaine levels in Sprague–Dawley rats preinjected with either saline, ethanol, or cocaine alone (5,10,22,33,35,41). In addition, our cocaine levels in blood and brain are similar in range to those ascribed in Lewis and Fischer rats (4,19). It is important to note that comparisons in blood, liver, and brain cocaine levels across the cited studies should be viewed with caution because of differences in experimental design(s), length of cocaine administration, and more importantly, because cocaine was administered alone and not in conjunction with ethanol. In this respect, this study is, to our knowledge, the first to use a combined ethanol–cocaine chronic regimen to ascertain the bioavailability of both cocaine and cocaethylene in liver, blood, and brain from different rat strains.

Broad substrate enzymes like catalase and esterase were measured in Long–Evans and Sprague–Dawley rats to demonstrate a significant relationship to ethanol–cocaine metabolism. This is of importance because differences in the activity of these enzymes could alter the behavioral response to ethanol or cocaine. Data obtained from this study, however, failed to show differences in enzymatic activity between Long–Evans and Sprague–Dawley rats. Although there are reports of both higher catalase activity in ethanol-preferring rats and positive correlations between aldehyde dehydrogenase activity and ethanol intake in Long–Evans and Sprague–Dawley rats (16), our results indicate that differences in ethanol drinking cannot be accounted for by differences in liver catalase activity. However, we did find that catalase and esterase activities were markedly suppressed in both strains when compared with appropriate control rats. Considering that in the present study a combined ethanol–cocaine regimen was implemented, liver enzymatic activity reported here must be interpreted with caution because all previous studies have only measured catalase or esterase activity in response to either ethanol or cocaine administration. This study points out the complexity of individual differences to polysubstance abuse, and suggests that further pharmacokinetic studies should help clarify the relative contribution(s) of catalase and esterase activity to possible etiologies and correlates of drug sensitivity.

In conclusion, the present study further supports the premise that Long–Evans and Sprague–Dawley rat strains exhibit differences in response to psychoactive drugs. Some of these variations include differences in drinking patterns to ethanol, behavioral activity to cocaine, and distribution of the active metabolite, cocaethylene. The fact that these differences are found in two commonly used rat strains suggests that these animals could be used to study the etiologies and correlates for susceptibility to substance abuse. Because outbred rats offer a less stable genotype than inbred rats strains, using Long–Evans and Sprague–Dawley phenotypes provide a more realistic model to examine interindividual differences to drug addiction such as those observed in heterologous humans.

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