Cocaine and Alcohol Interactions in the Rat: Effect of Cocaine and Alcohol Pretreatments on Cocaine Pharmacokinetics and Pharmacodynamics

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Abstract ☐ This experiment was designed to investigate the effect of pretreatment with cocaine and alcohol on cocaine pharmacokinetics and pharmacodynamics. Four groups of rats ($n = 8$ per group) received one of the following pretreatments for two weeks: none, alcohol (10% v/v in drinking water), cocaine (15 mg/kg/day ip), and alcohol+cocaine (10% v/v in drinking water+15 mg/kg/day ip). On the day of the experiment, cocaine was administered (30 mg/kg, ip) to each rat, either alone or in combination with alcohol (5 g/kg, po), in a balanced crossover experimental design. Plasma and brain ECF concentrations of cocaine and its three metabolites: benzoylecgonine, norcocaine, and cocaethylene were assayed by HPLC–UV. The percent change in brain dopamine concentration, mean arterial blood pressure, and heart rate were determined simultaneously. A sigmoid-E_{max} model was used to describe the brain cocaine concentration–neurochemical effect (dopamine) relationship, and an indirect pharmacodynamic response model was used to describe the plasma cocaine concentration–cardiovascular effect relationships. Alcohol pretreatment led to significant increase in cocaine AUC_p, $\alpha_{t1/2}$, and $\beta_{t1/2}$. Cocaine pretreatment significantly increased cocaine bioavailability, absorption rate constant, TBC, and the formation clearance of cocaethylene. Acute alcohol coadministration with cocaine increased cocaine AUC_p and bioavailability, reduced the fraction of cocaine dose converted to benzoylecgonine, and increased the formation of norcocaine. These results indicate that the pharmacokinetics of cocaine, either administered alone or in combination with alcohol, is significantly altered due to prior cocaine and/or alcohol use. Both cocaine and alcohol pretreatments increased the E_{max} for dopamine, with no effect on the EC_{50}. Acute alcohol coadministration with cocaine significantly increased the E_{max} for dopamine and reduced the EC_{50}. Cocaine pretreatment significantly decreased the I_{max} for blood pressure, IC_{50}, and R_{max}. For the heart rate response, both alcohol and cocaine pretreatments significantly increased the IC_{50}, with no effect on I_{max}. These results indicate that both cocaine and alcohol pretreatments as well as acute alcohol coadministration lead to significant alterations in cocaine pharmacodynamics that are due, at least in part, to the changes in cocaine pharmacokinetics. If similar effects occur in humans, chronic cocaine and alcohol abusers may respond differently to cocaine administration compared to naïve users and may be at higher risks of cocaine central nervous system toxicity.

Introduction

Concomitant cocaine and alcohol abuse has been associated with increased incidence of cocaine-related morbidity and mortality.[1] Studies in humans showed that alcohol consumption with cocaine led to significantly higher plasma cocaine and norcocaine concentrations and the formation of the pharmacologically active metabolite cocaethylene, whereas the concentrations of benzoylecgonine and ecgonine methyl ester were reduced.[2] In animal experiments, alcohol coadministration increased the plasma and brain cocaine concentrations and modified cocaine metabolic profile similar to what was observed in humans.[3,4] Results obtained from awake rats demonstrated that the changes in the neurochemical and cardiovascular responses to cocaine when administered with alcohol can be explained, at least partially, by the changes in cocaine pharmacokinetics and the contribution of cocaine metabolites to the pharmacological effects of cocaine.[5,6] These findings clearly indicate that factors that can alter cocaine pharmacokinetics and metabolic profile can lead to changes in the neurochemical and cardiovascular responses to cocaine administration.

Prior exposure to cocaine and alcohol has also been shown to alter cocaine pharmacokinetics, pharmacodynamics, and toxicities. We have reported previously that pretreating Wistar rats with 10% alcohol in drinking water for two weeks significantly slows the elimination rate of both cocaine and cocaethylene.[3] Several studies have demonstrated that 2−3 weeks of alcohol consumption enhances striatal [^{3}H]dopamine release and D_2 receptor binding in both the nucleus accumbens (N ACC) and striatum.[7−9] A human study has shown that alcohol pretreatment increases the preference for cocaine over monetary reinforcement, and that combined cocaine and alcohol abuse increases the risk of cardiac toxicity compared to cocaine alone.[10] In male mice, alcohol pretreatment in liquid diet for 5 days potentiates cocaine-induced hepatotoxicity, an effect dependent on the induction of the hepatic cytochrome P-450 mixed function oxidases.[11] Similar effects have also been observed in humans.[12] Cocaine absorption from the abdominal cavity to the systemic circulation after ip administration to rats is significantly faster after repeated cocaine administration.[13] Intermittent cocaine pretreatment for 1−9 days to laboratory animals augments brain extracellular fluid (ECF) dopamine, including that of the N ACC, which leads to enhancement of locomotor activity and stereotypy (i.e., sensitization).[14−16] In vitro studies in rodents have shown that cocaine pretreatment markedly induces cytochrome P-450 enzymes that are responsible for norcocaine formation.[17,18] Results from these investigations indicate that cocaine pharmacokinetics in individuals who have used cocaine and/or alcohol previously may be different from that in naïve users. This implies that alcoholics and cocaine addicts may respond differently to cocaine administration, and this may have significant clinical implications.

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The primary objective of this study was to investigate the effect of alcohol, cocaine, and combined alcohol and cocaine pretreatments on the pharmacokinetic and pharmacodynamic interactions between cocaine and alcohol. This was achieved by studying cocaine absorption, brain distribution, elimination, and metabolism when cocaine was given alone and in combination with alcohol to rats that were pretreated with alcohol, cocaine, or cocaine+ alcohol. The neurochemical and cardiovascular responses to cocaine administration were monitored simultaneously during the pharmacokinetic studies. The neurochemical response was assessed by determining the changes in brain N ACC dopamine level, while the cardiovascular responses were monitored by measuring the changes in the mean arterial blood pressure and heart rate. The information obtained from this study can help to identify and predict pharmacokinetic factors that may lead to increased risks of toxicity with combined cocaine and alcohol abuse. This is the first report to investigate the effect of pretreatment with cocaine and alcohol on cocaine pharmacokinetics and pharmacodynamics. The possible interactions among these pretreatments and acute alcohol coadministration were also examined.

Materials and Methods

Chemicals and Reagents—Cocaine hydrochloride and cocaethylene hydrochloride were purchased from Research Biochemicals International (Natick, MA). Bupivacaine and sodium fluoride were obtained from Sigma Chemical (St. Louis, MO). Chloroform was supplied by Burdick and Jackson Laboratory (Muskegon, MI). The dehydrated 200 proof ethyl alcohol (USP) was purchased from McCormick Distilling (Weston, MO). All solvents were of high performance liquid chromatographic (HPLC) grade, and all chemicals were of analytical reagent (AR) grade.

Cocaine and Alcohol Pretreatments—Male Wistar rats (250−350 g, Simonsen Laboratories, Gilroy, CA) were maintained one per cage on a 12-h light/dark cycle with Purina chow pellets and water ad libitum for 7 days before use in the experiments. Water and food consumption and body weight were recorded daily. Thirty-two rats were assigned randomly to one of the following four pretreatment groups (n = 8 rats per group): control (no pretreatment), alcohol pretreatment, cocaine pretreatment, and combined cocaine and alcohol pretreatment. For the alcohol pretreatment group, the rats were allowed free access to 10% alcohol in water (v/v) as their sole source of drinking water. Cocaine pretreatment was accomplished by injecting the rats with 15 mg/kg cocaine ip once daily for 14 days, and the rats were allowed free access to drinking water. In the combined cocaine and alcohol pretreatment group, the rats were allowed free access to 10% alcohol in water (v/v) as their sole source of drinking water and were injected with 15 mg/kg cocaine ip once daily for 14 days. During the entire two weeks of pretreatments, the rats in all groups were maintained one per cage on a 12-h light/dark cycle with Purina chow pellets ad libitum. Water and food consumption and rat body weight were recorded daily. The pretreatment duration (14-day) and the daily doses of cocaine (15 mg/kg/day, ip) and alcohol (~3 g/kg/day, po) for the pretreatments were chosen based on the results of previous studies.[3,9,13−15,19]

Animal Care and Preparation—All animal preparation procedures were in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health Publication No. 85-23, revised 1985) and were approved by the institutional animal care and use committee at Washington State University. Details of the animal preparation procedures were described previously.[20] Briefly, after 7 days of pretreatment, the brain microdialysis guide cannula was implanted in the rat brain followed, 7 days later, by femoral vein and artery cannulation, and abdominal and gastric catheter implantation. The brain microdialysis probe was inserted into the guide cannula to replace the dummy probe, and the targeted area was the N ACC. The rats from each of the four pretreatment groups were given 30 mg/kg ip cocaine alone and in combination with 5 g/kg alcohol in a crossover experimental design with 48-h washout period between treatments.

Pharmacokinetic and Pharmacodynamic Studies—On the day of the experiment, one of the femoral artery cannulae was connected to a pressure transducer linked to a blood pressure analyzer (Digi-Med Model 190, Micro-Med, Louisville, KY) for monitoring the mean arterial blood pressure and heart rate. The signals from the analyzer were collected, updated, and averaged every one minute by a system integrator (Digi-Med Model 200, Micro-Med, Louisville, KY) and were stored in a computer for subsequent analysis. Meanwhile, the brain microdialysis effluent was collected every 20 min (at 1 μL/min) into HPLC autosampler vials containing 20 μL of dopamine mobile phase and vortex-mixed. Five microliters of this mixture was injected immediately into an HPLC equipped with an electrochemical (EC) detector for dopamine analysis. Once a stable dopamine baseline was achieved, the rats were treated with either 10 g/kg normal saline or 5 g/kg alcohol (50% v/v in normal saline) through the gastric catheter. Twenty minutes later, 30 mg/kg of cocaine was administered through the abdominal catheter. After drug administration, 10 blood samples, each of 0.2 mL were collected through the femoral artery cannula at 2, 5, 10, 15, 30, 60, 90, 120, 180, 240 min in heparin and sodium fluoride pretreated vacutainers to avoid cocaine and cocaethylene hydrolysis by plasma carboxylesterases. Plasma samples were obtained by centrifugation and were stored at −20 °C until analyzed for cocaine and its metabolites by HPLC with ultraviolet (UV) detection. The effluent of the microdialysis probe was continuously collected every 20 min throughout the experiment into HPLC autosampler vials containing 20 μL of dopamine mobile phase (pH 4). The purpose of this treatment was to maintain dopamine, cocaine, and its metabolites under acidic condition to reduce their spontaneous oxidation and hydrolysis. After mixing the vial content, 5 μL was injected immediately into the HPLC-EC system for dopamine analysis, and the rest was analyzed for cocaine and its metabolites by HPLC−UV. The mean arterial blood pressure and the heart rate were continuously monitored during the entire experiment.

After the above two treatments, the rats from each of the four pretreatment groups received cocaine iv (6.8 mg/kg) and cocaethylene iv (3.9 mg/kg) through the femoral vein cannula with a 24-h washout period between the treatments. The purpose of the iv cocaine administration was to determine the systemic bioavailability for ip cocaine. The pharmacokinetic parameters for cocaethylene obtained after iv administration in this study and those for benzoylecgonine and norcocaine obtained from one of our previous studies were used to determine the effect of alcohol on cocaine metabolic profile.[6] This was accomplished by comparing the fraction of cocaine dose converted to each of the metabolites when ip cocaine was administered either alone or in combination with alcohol, in each of the pretreatment groups.

Analytical Methods—*Cocaine and Its Metabolites*—Plasma and microdialysis probe effluent were analyzed for cocaine and its metabolites using the method developed in our laboratory.[21] The actual concentrations of cocaine and its metabolites in the brain ECF were determined from the probe effluent concentration after correcting for the probe recovery which was determined from an in vitro calibration experiment.[22]

Dopamine—The microdialysis probe effluent was injected directly into an HPLC system equipped with EC detector for dopamine analysis immediately after collection. Details of the analytical procedures used for dopamine determination in the microdialysis probe effluent were described previously.[20]

Pharmacokinetic Analysis—A two-compartment pharmacokinetic model with elimination from the central compartment was used to investigate the effect of acute alcohol coadministration, and alcohol or cocaine pretreatment on cocaine absorption, distribution, and elimination after ip cocaine administration. Cocaine pharmacokinetic parameters were estimated by fitting cocaine plasma and brain ECF concentrations to the two equations that describe cocaine concentration−time profile in plasma and in the brain ECF simultaneously utilizing PCNONLIN 4.0 (SCI Software, Lexington, KY).[5,6] The bioavailability of cocaine after ip administration was calculated from the corresponding areas under the plasma concentration−time curves (AUC_p) after ip and iv cocaine administrations to each rat. The fraction of the ip cocaine dose converted to each of the metabolites was calculated as described previously.[6]

Pharmacodynamic Analysis—The sigmoid-E_{max} pharmacodynamic model was used to describe the brain ECF cocaine concentration−neurochemical response relationship. The phar-

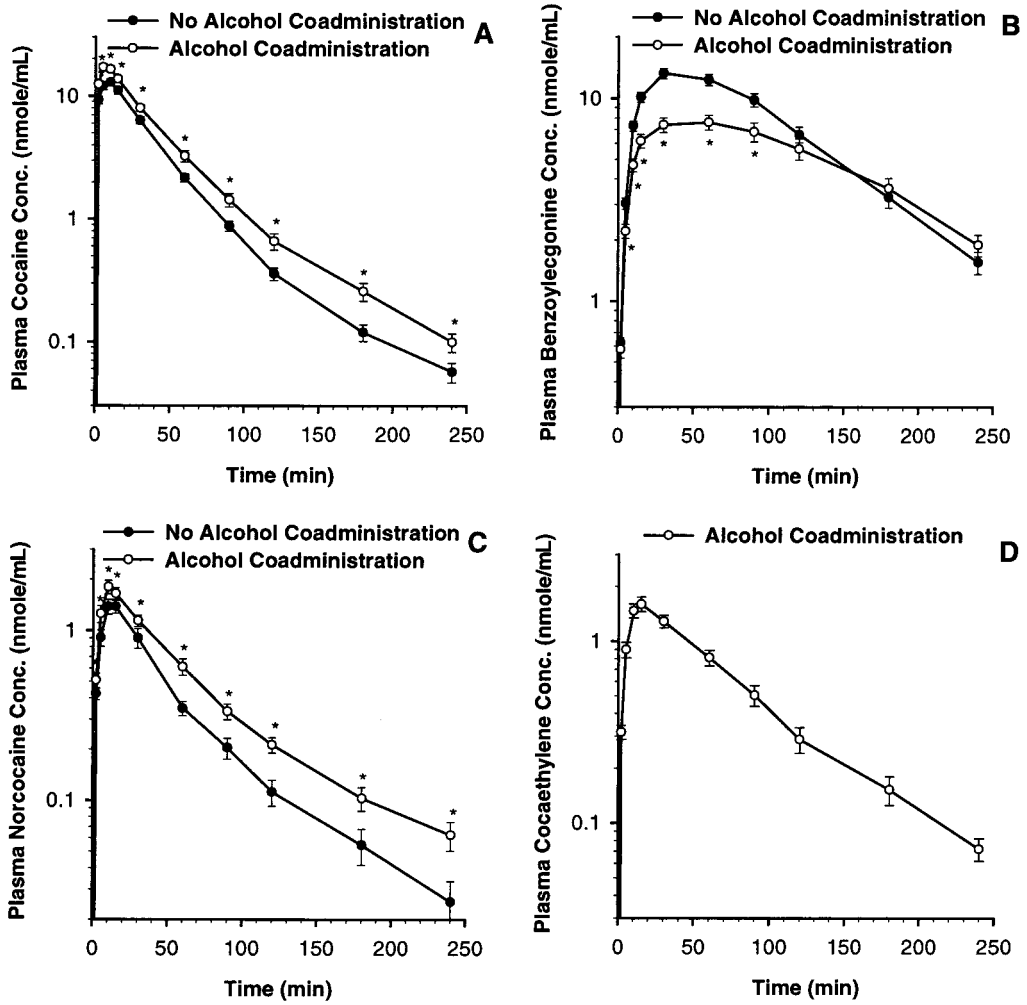


Figure 1—Plasma concentration–time profiles of cocaine (A), benzoylecgonine (B), norcocaine (C), and cocaethylene (D) after 30 mg/kg ip cocaine challenge to rats that were not (●) and were (○) coadministered with 5 g/kg acute alcohol. Each data point is presented as mean ± SE ($n = 32$). *Significantly different from that of no alcohol coadministration ($p < 0.05$).

macodynamic model parameters were estimated by fitting the percent change in dopamine brain ECF concentration and the cocaine brain ECF concentration to the model equation utilizing PCNONLIN. The relationship between plasma cocaine concentration and the change in mean arterial blood pressure and heart rate after ip cocaine administration was characterized by an indirect mechanism-based pharmacodynamic response model.[5,23] The mathematical expression that describes the relationship between the change in the pharmacological response and the drug concentration is:

$$\frac{dR}{dt} = k_{in} - k_{out}\left(1 - \frac{I_{max}C_p^n}{IC_{50}^n + C_p^n}\right)R \qquad (1)$$

where R is the observed response (percent change in mean arterial blood pressure or heart rate), k_{in} is the apparent zero-order rate constant for response production, k_{out} is the first-order rate constant for response dissipation, I_{max} is the maximum inhibition of the factor that produces the effect, IC_{50} is the plasma cocaine concentration that leads to 50% inhibition of the factor that produces the effect, C_p is the plasma cocaine concentration at the time of the observed response, and n is the sigmoidicity factor.[5,6] The maximum response that will be achieved as the ip dose is very high or IC_{50} approaches zero is:

$$R_{max} = \frac{R_0}{1 - I_{max}} \qquad (2)$$

where R_{max} is the maximum response and R_0 is the basal response (100%).

The indirect pharmacodynamic model parameters were estimated by fitting the percent change in mean arterial blood pressure or heart rate and the plasma cocaine concentration at different time points to eq 1. Nonlinear regression analysis was performed using PCNONLIN as detailed previously.[5,6]

Statistical Analysis—In this study, the control, alcohol, cocaine, and cocaine+alcohol pretreatments, together with ip cocaine administration (with and without acute alcohol coadministration), represented a four-way factorial experiment ($2 \times 2 \times 2 \times 2$) with repeated measures on the acute alcohol treatment factor. This experimental design allowed studying the effect of pretreatment with alcohol and cocaine and the effect of acute alcohol coadministration on the pharmacokinetics and pharmacodynamics of cocaine. The subgroup differences were partitioned within the analysis of variance (ANOVA) structure when examining the effect of one of the three main factors. The significance of the interaction between the two pretreatment factors (cocaine+alcohol pretreatment) and their interaction with acute alcohol coadministration factor could also be determined with the analysis of variance. Note that testing interaction effects is equivalent to testing whether there is synergism between the two treatments. Because no measurement for heart rate was made for the control (i.e., naïve) group, the analysis of variance for this response was conducted assuming no interactions between treatment factors. The statistical analyses of the estimated pharmacokinetic and pharmacodynamic parameters of cocaine and its metabolites were performed using the Statistical Analysis System Release 6.12 (SAS Institute Inc., Cary, NC). Multiple comparisons with Bonferroni correction were conducted to examine the selected statistical differences between treatments. A difference of $p < 0.05$ was considered statistically significant.

Table 1—Pharmacokinetic Parameters of Cocaine and Its Metabolites in the Rat Either with or without Alcohol or Cocaine Pretreatments or Acute Alcohol Coadministration (mean ± SE, $n = 32$)

pharmacokinetic parameter	alcohol pretreatment		cocaine pretreatment		alcohol coadministration	
	no	yes	no	yes	no	yes
			Cocaine			
AUC_p (nmol·min/mL)	528 ± 36	639 ± 39[a]	594 ± 46	573 ± 30	490 ± 29	677 ± 40[c]
AUC_b (nmol·min/mL)	605 ± 67	768 ± 62	751 ± 79	622 ± 47	634 ± 63	736 ± 68
AUC_b/AUC_p	1.152 ± 0.087	1.31 ± 0.11	1.36 ± 0.13	1.102 ± 0.060	1.325 ± 0.099	1.13 ± 0.10[c]
TBC (mL/min/kg)	125.0 ± 5.4	112.6 ± 5.0	107.7 ± 3.7	129.9 ± 6.0[b]	121.9 ± 5.4	115.6 ± 5.3
F	0.719 ± 0.043	0.762 ± 0.037	0.669 ± 0.035	0.812 ± 0.041[b]	0.65 ± 0.04	0.832 ± 0.033[c]
k_a (min^{-1})	0.361 ± 0.035	0.419 ± 0.039	0.306 ± 0.031	0.474 ± 0.037[b]	0.401 ± 0.042	0.378 ± 0.032
$\alpha_{t1/2}$ (min)	11.45 ± 0.88	16.45 ± 0.89[a]	13.5 ± 1.1	14.39 ± 0.92	15.07 ± 0.91	12.8 ± 1.0
$\beta_{t1/2}$ (min)	39.7 ± 3.2	66.5 ± 4.6[a]	49.9 ± 5.2	56.4 ± 4.0	52.0 ± 4.6	54.3 ± 4.7
V_c (L/kg)	2.84 ± 0.18	3.34 ± 0.17[a]	2.69 ± 0.18	3.49 ± 0.15[b]	3.13 ± 0.19	3.05 ± 0.17
$V_{d\beta}$ (L/kg)	7.21 ± 0.74	10.62 ± 0.83[a]	7.39 ± 0.71	10.43 ± 0.88[b]	9.2 ± 1.0	8.63 ± 0.64
			Benzoylecgonine			
AUC_p (nmol·min/mL)	1620 ± 170	1560 ± 120	1710 ± 170	1460 ± 120	1740 ± 120	1440 ± 160[c]
AUC_b (nmol·min/mL)	196 ± 25	200 ± 29	245 ± 33	151 ± 16[b]	226 ± 31	170 ± 21[c]
AUC_b/AUC_p	0.125 ± 0.012	0.128 ± 0.015	0.149 ± 0.016	0.1034 ± 0.0083[b]	0.128 ± 0.015	0.125 ± 0.011
f_m	0.270 ± 0.028	0.259 ± 0.020	0.285 ± 0.028	0.244 ± 0.020	0.289 ± 0.020	0.240 ± 0.027[c]
f_m·TBC (mL/min/kg)	32.3 ± 2.7	30.4 ± 2.5	30.0 ± 2.3	32.7 ± 2.9	34.7 ± 2.7	28.0 ± 2.4[c]
			Norcocaine			
AUC_p (nmol·min/mL)	83.0 ± 9.6	99.4 ± 8.7	89 ± 10	93.9 ± 7.9	74.3 ± 8.2	108.1 ± 9.3[c]
AUC_b (nmol·min/mL)	57 ± 12	52.7 ± 4.9	60 ± 12	50.1 ± 4.6	52 ± 10	58.0 ± 7.6
AUC_b/AUC_p	0.631 ± 0.067	0.642 ± 0.076	0.687 ± 0.089	0.585 ± 0.048	0.713 ± 0.083	0.559 ± 0.055
f_m	0.122 ± 0.014	0.146 ± 0.013	0.131 ± 0.015	0.137 ± 0.012	0.110 ± 0.012	0.159 ± 0.014[c]
f_m·TBC (mL/min/kg)	15.1 ± 1.5	16.4 ± 1.5	13.4 ± 1.4	18.1 ± 1.5[b]	12.7 ± 1.3	18.8 ± 1.5[c]
			Cocaethylene			
AUC_p (nmol·min/mL)	114 ± 11	137 ± 10	129 ± 13	121.7 ± 8.3		126 ± 11
AUC_b (nmol·min/mL)	86 ± 16	102 ± 11	98 ± 17	90.3 ± 9.7		94 ± 13
AUC_b/AUC_p	0.730 ± 0.091	0.807 ± 0.098	0.78 ± 0.12	0.759 ± 0.064		0.768 ± 0.094
f_m	0.280 ± 0.040	0.210 ± 0.022	0.186 ± 0.034	0.303 ± 0.052[b]		0.245 ± 0.033
f_m·TBC (mL/min/kg)	38.4 ± 6.7	25.8 ± 3.4	20.3 ± 4.3	43.9 ± 9.0[b]		32.1 ± 5.3

[a] Significantly different from no alcohol pretreatment ($p < 0.05$, $F_{1,24}$). [b] Significantly different from no cocaine pretreatment ($p < 0.05$, $F_{1,24}$). [c] Significantly different from no acute alcohol coadministration ($p < 0.05$, $F_{1,24}$).

Results

During the pretreatment period, the rats in alcohol and cocaine+alcohol groups consumed 7.14 ± 0.58 mL/kg/day and 7.83 ± 0.43 mL/kg/day of alcohol, respectively. The surgical procedures for microdialysis guide cannula implantation caused slight reduction in water and food intakes; however, these values soon returned to their preoperative levels. Meanwhile, the rats in all groups gained weight steadily, and the rate of increase was 2.1 ± 1.8 g/day for the alcohol group, 3.2 ± 1.2 g/day for the cocaine group, and 2.49 ± 0.97 g/day for the cocaine+alcohol group. There were not any significant signs of malnutrition or changes in the general health of the rats during the pretreatment period as indicated by their steady body weight gain.

The plasma concentration−time profiles for cocaine and its metabolites after ip administration of cocaine alone and in combination with alcohol are presented in Figure 1. A summary of the pharmacokinetic parameters for cocaine and its metabolites after different pretreatments is listed in Table 1. Alcohol pretreatment caused significant increase in cocaine AUC_p ($p = 0.044$), without affecting area under the brain ECF concentration−time curve (AUC_b) and brain to plasma distribution ratio (AUC_b/AUC_p). Cocaine distribution half-life ($\alpha_{t1/2}$) was increased ($p = 0.0019$), elimination half-life ($\beta_{t1/2}$) was prolonged ($p = 0.0003$), and the volume of distribution of cocaine during the elimination phase ($V_{d\beta}$) was larger ($p = 0.011$) due to alcohol pretreatment. The systemic bioavailability of cocaine (F) significantly increased ($p = 0.026$) due to cocaine pretreatment. However, the increase in cocaine bioavailability was offset by the increase in cocaine total body clearance (TBC, $p = 0.037$), causing no significant changes in cocaine AUC_p, AUC_b, or AUC_b/AUC_p after cocaine pretreatment. Cocaine pretreatment also increased k_a ($p = 0.0043$), volume of distribution of the central compartment (V_c, $p = 0.0046$), and $V_{d\beta}$ ($p = 0.022$). Alcohol coadministration with cocaine caused significant increases in cocaine AUC_p ($p = 0.0005$) and F ($p = 0.0001$), and significant decrease in AUC_b/AUC_p, which was similar to what has been reported previously.[5] Alcohol coadministration significantly decreased cocaine TBC ($p = 0.041$) and acted synergistically with alcohol pretreatment to prolong $\beta_{t1/2}$ ($p = 0.0067$) and increase $V_{d\beta}$ ($p = 0.0047$).

Pretreatment with alcohol or cocaine did not have any significant effect on benzoylecgonine formation. However, these two pretreatments acted synergistically to significantly reduce the formation of benzoylecgonine ($p = 0.048$). Meanwhile, acute alcohol coadministration with cocaine significantly reduced benzoylecgonine AUC_p ($p = 0.021$), AUC_b ($p = 0.023$), f_m ($p = 0.021$), and formation clearance (f_m·TBC, $p = 0.0029$). Norcocaine formation clearance after cocaine administration was significantly increased due to cocaine pretreatment ($p = 0.042$), and the combined pretreatment with cocaine and alcohol significantly increased norcocaine formation ($p = 0.038$). Meanwhile, acute alcohol coadministration with cocaine led to significant increase in norcocaine AUC_p ($p = 0.0002$), f_m ($p = 0.0002$), and f_m·TBC ($p = 0.0001$). Cocaine pretreatment significantly increased cocaethylene f_m·TBC ($p = 0.020$). Even though alcohol pretreatment did not have any effect on cocaine metabolic conversion to cocaethylene, it acted synergistically with cocaine pretreatment to significantly increase the formation of cocaethylene ($p = 0.033$) and f_m·TBC ($p = 0.026$).

The brain ECF dopamine concentration−time profiles after cocaine administration with and without alcohol in

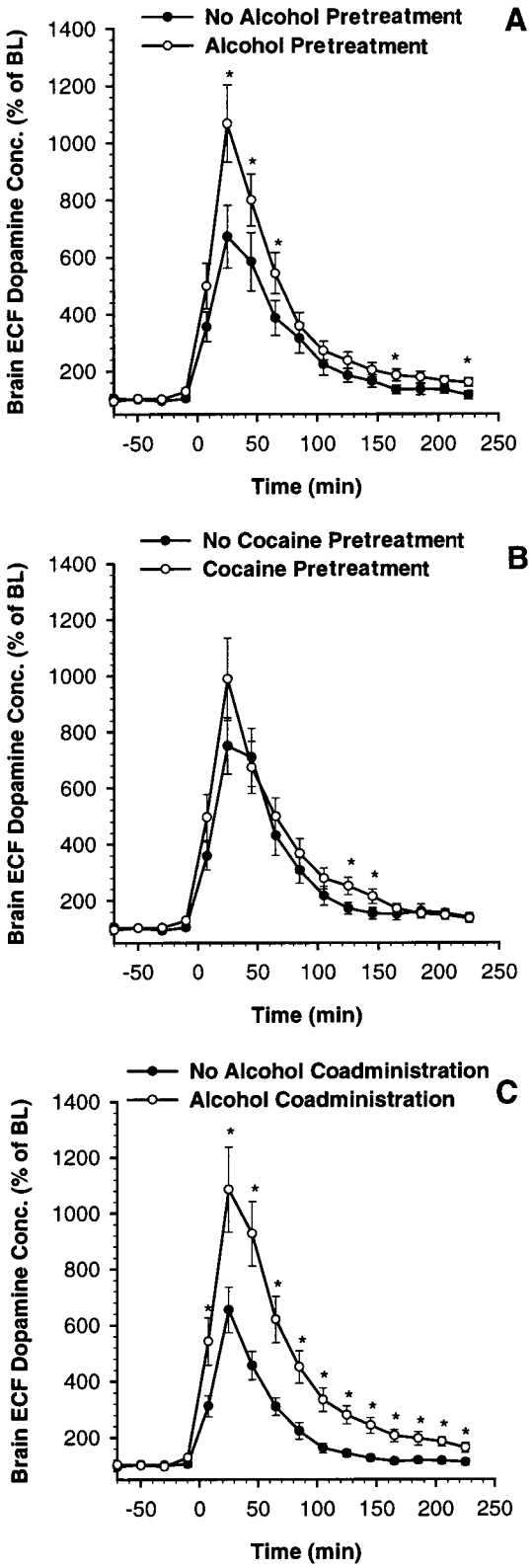


Figure 2—Brain ECF dopamine concentration–time profiles after 30 mg/kg ip cocaine challenge to (A) alcohol naive (●) and alcohol pretreated (○) rats. *Significantly different from that of alcohol naive rats ($p < 0.05$); (B) cocaine naive (●) and cocaine pretreated (○) rats. *Significantly different from that of cocaine naive rats ($p < 0.05$); (C) rats that were not (●) and were (○) coadministered with 5 g/kg acute alcohol. *Significantly different from that of no alcohol coadministration ($p < 0.05$). Each data point is presented as mean ± SE ($n = 32$). BL = Baseline.

the different pretreatment groups are shown in Figure 2. The brain ECF dopamine concentration increased rapidly after 30 mg/kg ip cocaine administration, and it gradually declined to its baseline value at the end of the 4-hour experiment period. Alcohol pretreated rats had significantly higher brain ECF dopamine concentrations in response to cocaine administration when compared to alcohol naïve rats. Cocaine pretreatment did not have any significant effects on dopamine levels after a cocaine challenge dose when compared with cocaine naïve rats. Alcohol coadministration with cocaine significantly increased the magnitude and duration of the brain ECF dopamine level augmentation when compared with cocaine administration alone. The estimated pharmacodynamic parameters of neurochemical response to cocaine are summarized in Table 2. Estimates of the sigmoid-E_{max} pharmacodynamic model parameters showed that alcohol and cocaine pretreatments increased the E_{max} for the neurochemical response to cocaine administration but these changes were not statistically significant. On the other hand, alcohol coadministration with cocaine caused significant increase in E_{max} ($p = 0.018$) and significant reduction in EC_{50} ($p = 0.014$).

The mean arterial blood pressure increased rapidly after ip cocaine challenge. It then declined slowly and did not return to its baseline value at the end of the 4-h experiment (Figure 3). Alcohol and cocaine pretreatments, as well as acute alcohol coadministration with cocaine, caused significant reduction in the mean arterial blood pressure elevation in response to cocaine administration. Since alcohol alone caused, on average, 5−10% decrease in blood pressure, we partitioned this effect from the combined effect of cocaine+alcohol and used the corrected values in the pharmacodynamic modeling.[5] The estimated pharmacodynamic parameters for the mean arterial blood pressure response to cocaine are summarized in Table 3. Analysis with the indirect pharmacodynamic inhibitory model showed that alcohol pretreatment caused reduction in IC_{50} ($p = 0.058$). Cocaine pretreatment significantly decreased the I_{max} ($p = 0.0012$), IC_{50} ($p = 0.0052$), and R_{max}, which is the maximum response to cocaine administration ($p = 0.0041$). Alcohol and cocaine pretreatments did not have any effect on the onset and dissipation rate constants of the blood pressure response. However, alcohol and cocaine pretreatments acted synergistically to reduce the IC_{50} for the pressor response ($p = 0.029$). On the other hand, acute alcohol coadministration increased the rate constant for blood pressure response production (k_{in}) ($p = 0.0093$) and its dissipation (k_{out}) ($p = 0.0085$).

The heart rate decreased rapidly after cocaine ip administration and neither alcohol nor cocaine pretreatments had any significant effect on the change in heart rate in response to cocaine administration (Figure 4A,B). Meanwhile, cocaine caused more reduction in heart rate when it was administered with alcohol (Figure 4C), even though alcohol administration alone led to increase in heart rate (data not shown). Because alcohol alone caused, on average, 10−20% increase in heart rate, we partitioned this effect from the combined effect of cocaine+alcohol, and the corrected values were used in the pharmacodynamic modeling. The estimated pharmacodynamic parameters for the heart rate response to cocaine administration are also summarized in Table 3. Alcohol and cocaine pretreatments caused significant increase in IC_{50} ($p = 0.0001$ and 0.0017, respectively). On the other hand, acute alcohol coadministration increased the rate constant for the heart rate response production (k_{in}) ($p = 0.032$) and its dissipation (k_{out}) ($p = 0.065$).

Discussion

Illicit drugs are mostly consumed by addicts who often abuse multiple drugs simultaneously. This observation

Table 2—Pharmacodynamic Parameters for the Neurochemical Response to Cocaine in the Rat Either with or without Alcohol or Cocaine Pretreatments or Acute Alcohol Coadministration (mean ± SE, $n = 32$)

pharmacodynamic parameter	alcohol pretreatment		cocaine pretreatment		alcohol coadministration	
	no	yes	no	yes	no	yes
E_{max} (% of baseline)	1100 ± 200	1530 ± 240	1160 ± 190	1480 ± 250	980 ± 140	1650 ± 270[a]
EC_{50} (nmol/mL)	9.0 ± 1.1	8.57 ± 0.85	8.38 ± 0.86	9.2 ± 1.1	10.35 ± 0.94	7.26 ± 0.96[a]
n	1.86 ± 0.31	1.69 ± 0.30	1.96 ± 0.30	1.60 ± 0.31	1.94 ± 0.40	1.62 ± 0.16

[a] Significantly different from no acute alcohol coadministration ($p < 0.05$, $F_{1,24}$).

Table 3—Pharmacodynamic Parameters for the Mean Arterial Blood Pressure and Heart Rate Response to Cocaine in the Rat Either with or without Alcohol or Cocaine Pretreatments or Acute Alcohol Coadministration (mean ± SE, $n = 32$)

pharmacodynamic parameter	alcohol pretreatment		cocaine pretreatment		alcohol coadministration	
	no	yes	no	yes	no	yes
			Mean Arterial Blood Pressure			
k_{in} (% of baseline/min)	30.9 ± 5.3	31.0 ± 5.0	27.7 ± 4.5	34.2 ± 5.6	20.6 ± 2.3	41.2 ± 6.3[c]
k_{out} (min^{-1})	0.283 ± 0.050	0.310 ± 0.054	0.257 ± 0.043	0.336 ± 0.059	0.191 ± 0.022	0.402 ± 0.065[c]
I_{max}	0.253 ± 0.018	0.239 ± 0.015	0.287 ± 0.014	0.205 ± 0.014[b]	0.232 ± 0.015	0.260 ± 0.017
IC_{50} (nmol/mL)	13.0 ± 2.0	9.2 ± 1.2	14.0 ± 1.9	8.1 ± 1.1[b]	11.5 ± 1.9	10.7 ± 1.3
R_{max} (% of baseline)	136.5 ± 3.7	133.1 ± 2.9	142.3 ± 3.2	127.3 ± 2.9[b]	132.0 ± 2.9	137.6 ± 3.6
			Heart Rate			
k_{in} (% of baseline/min)	41.7 ± 5.6	47.5 ± 5.6	43.5 ± 8.7	46.6 ± 4.6	35.2 ± 6.3	56.0 ± 4.7[c]
k_{out} (min^{-1})	0.442 ± 0.056	0.472 ± 0.055	0.436 ± 0.086	0.475 ± 0.045	0.374 ± 0.066	0.550 ± 0.043
I_{max}	0.270 ± 0.021	0.273 ± 0.015	0.291 ± 0.017	0.263 ± 0.015	0.260 ± 0.024	0.284 ± 0.011
IC_{50} (nmol/mL)	1.48 ± 0.30	7.3 ± 1.2[a]	4.8 ± 1.1	5.7 ± 1.2[b]	6.4 ± 1.5	4.3 ± 1.0
R_{max} (% of baseline)	138.7 ± 4.2	139.6 ± 3.2	142.3 ± 3.6	137.8 ± 3.4	137.8 ± 5.3	140.7 ± 2.3

[a] Significantly different from no alcohol pretreatment ($p < 0.05$, $F_{1,24}$). [b] Significantly different from no cocaine pretreatment ($p < 0.05$, $F_{1,24}$). [c] Significantly different from no acute alcohol coadministration ($p < 0.05$, $F_{1,24}$).

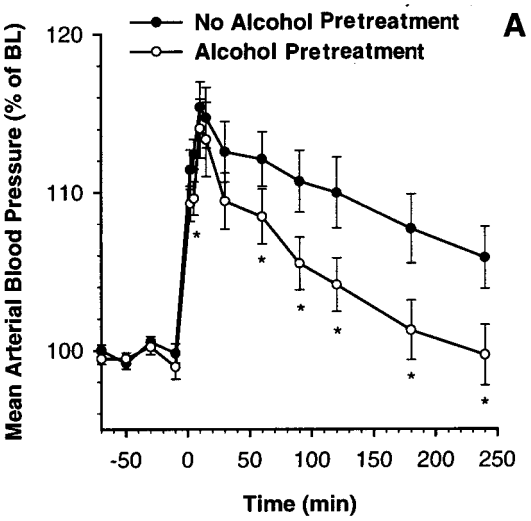
clearly illustrates the importance of studying possible interactions between illicit drugs and changes in the disposition and pharmacological effects of these substances of abuse when two or more of them are consumed simultaneously. Most of the studies conducted thus far involve administration of a single drug in naïve animals or in subjects that had been abstinent for several months from drugs. Our investigation was designed to study how prior cocaine and alcohol use, acute alcohol coadministration, and interactions among these factors can affect the pharmacokinetics and pharmacodynamics of cocaine.

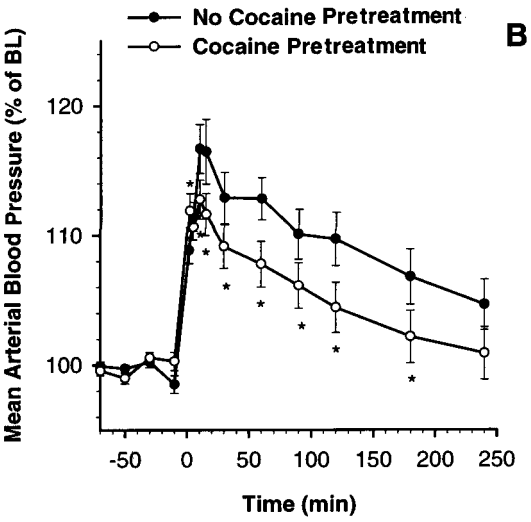
Alcohol pretreatment for two weeks significantly prolonged cocaine distribution and elimination half-lives and significantly increased cocaine volume of distribution. Similar effect on cocaine elimination half-life has been reported previously in anesthetized rats.[3] Cocaine AUC_p increased significantly after alcohol pretreatment, which may have resulted from the inhibition of cocaine metabolism because its bioavailability did not change significantly. Alcohol metabolism in mammals is mediated by cytosolic alcohol dehydrogenase, microsomal CYP2E1, and peroxisomal catalase.[24] CYP2E1 catalyzes the biotransformation of a variety of endogenous and exogenous compounds and is inducible by alcohol. It is possible that CYP2E1 induction by alcohol pretreatment may be involved in the changes in cocaine metabolism and pharmacokinetics. Studies have shown that both acute and chronic alcohol administration decrease hepatic glutathione content and shift its reduction vs oxidation ratio to an unfavorable condition for the cell.[25] Another effect related to alcohol pretreatment is the promotion of lipid peroxidation in the liver.[26] These two CYP2E1-dependent events can lead to intracellular oxidative stress and slow the liver biotransformation activity. These results imply that prior use of alcohol can lead to higher and prolonged cocaine plasma concentrations after administration of the same cocaine dose which may, in turn, increase cocaine-related toxicities.

We pretreated the rats with ip cocaine administration because it is easier than iv and more drug will reach the systemic circulation compared to oral and subcutaneous administration. Cocaine pretreatment significantly increased the rate and extent of cocaine absorption after an ip challenge dose of cocaine. However, the increase in cocaine bioavailability was not accompanied by an increased in AUC_p and AUC_b. This may be due to the significant increase in cocaine TBC. Cocaine pretreatment also increased cocaine volume of distribution which may explain why its elimination half-life was not affected by cocaine pretreatment.

Acute alcohol coadministration with cocaine significantly increased cocaine AUC_p and systemic bioavailability. Meanwhile, cocaine $\beta_{t1/2}$ and TBC were not significantly different, indicating that the increase in cocaine AUC_p was primarily due to the increase in cocaine bioavailability. The enhanced cocaine systemic bioavailability may be caused by the inhibitory effect of alcohol on cocaine presystemic metabolism which resulted in a larger fraction of the cocaine dose escaping the first-pass metabolism and reaching the systemic circulation. Examination of the significant interactions between different factors showed that acute alcohol and alcohol pretreatment acted synergistically to prolong cocaine elimination half-life and volume of distribution. The increase in the plasma cocaine concentration after alcohol coadministration with cocaine was accompanied by less than proportional increase in cocaine AUC_b and thus caused decrease in AUC_b/AUC_p. We have previously demonstrated that in the presence of much higher plasma alcohol concentration, brain ECF cocaine concentration was significantly increased in rats.[4] These results showed that combined alcohol and cocaine use can lead to higher cocaine concentrations, which may augment cocaine pharmacological effects and toxicities.

Alcohol pretreatment did not have any significant effect on cocaine metabolic profile. However, cocaine pretreatment significantly reduced benzoylecgonine formation and significantly increased the formation of the active metabolite cocaethylene. The increased formation of cocaethylene may have significant pharmacological and toxicological





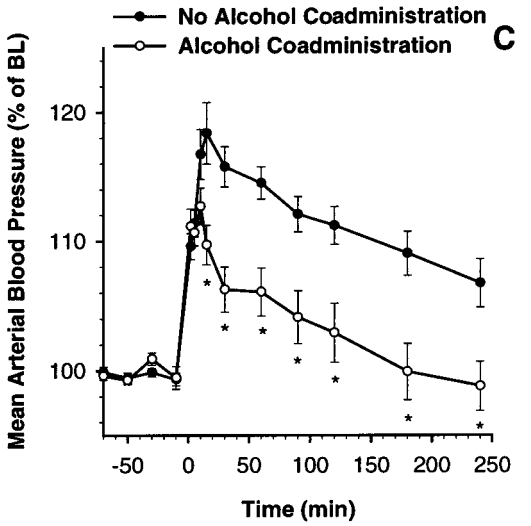
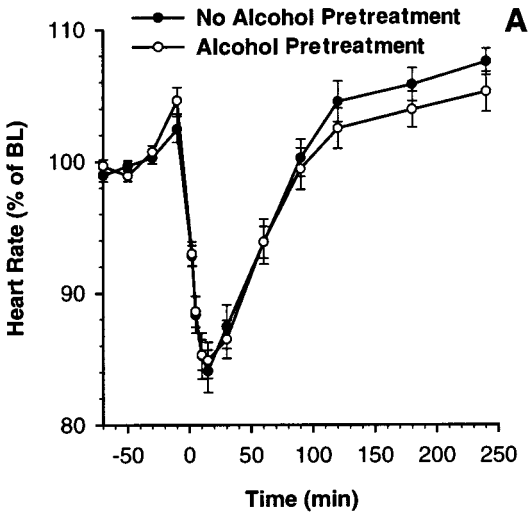
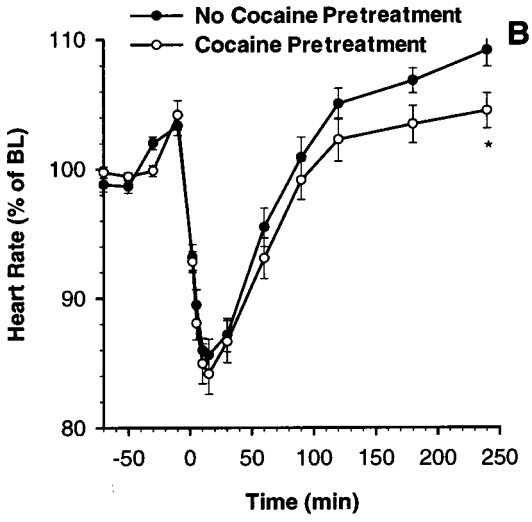


Figure 3—Mean arterial blood pressure–time profiles after 30 mg/kg ip cocaine challenge to (A) alcohol naive (●) and alcohol pretreated (○) rats. *Significantly different from that of alcohol naive rats ($p < 0.05$); (B) cocaine naive (●) and cocaine pretreated (○) rats. *Significantly different from that of cocaine naive rats ($p < 0.05$); (C) rats that were not (●) and were (○) coadministered with 5 g/kg acute alcohol. *Significantly different from that of no alcohol coadministration ($p < 0.05$). Each data point is presented as mean ± SE ($n = 32$). BL = Baseline.





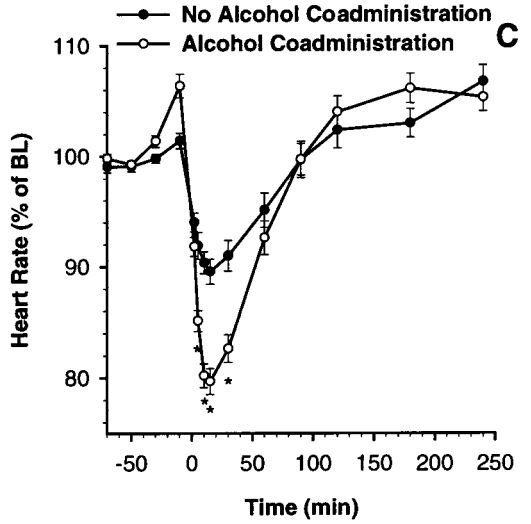


Figure 4—Heart rate–time profiles after 30 mg/kg ip cocaine challenge to (A) alcohol naive (●) and alcohol pretreated (○) rats; (B) cocaine naive (●) and cocaine pretreated (○) rats. *Significantly different from that of cocaine naive rats ($p < 0.05$); (C) rats that were not (●) and were (○) coadministered with 5 g/kg acute alcohol. *Significantly different from that of no alcohol coadministration ($p < 0.05$). Each data point is presented as mean ± SE ($n = 32$). BL = Baseline.

consequences. This is because, compared to cocaine, cocaethylene has been shown to be more potent with respect to the neurochemical, heart rate, and QRS interval responses and is equipotent in causing mean arterial blood

pressure increase.[6] Cocaine and alcohol coadministration significantly reduced benzoylecgonine formation, with approximately 25% of cocaine dose converted to the active metabolite cocaethylene. The fact that alcohol coadministration caused only 5% reduction in the precent of the cocaine dose converted to benzoylecgonine, but approximately 25% of cocaine dose was converted to cocaethylene indicates that cocaethylene formation is not solely on the expense of benzoylecgonine formation. These results imply that alcohol may also affect the metabolism of cocaine to ecgonine methyl ester. Another significant effect of acute alcohol coadministration on cocaine metabolism was the significant increase in the formation of norcocaine. On the other hand, cocaine+alcohol pretreatment led to more than additive effect on the reduction of benzoylecgonine and increase of norcocaine formation. The changes in cocaine metabolic profile due to alcohol coadministration, specifically the formation of cocaethylene and the increased formation of norcocaine, can significantly alter cocaine pharmacological and toxicological effects because of the contribution of these two metabolites to the neurochemical and cardiovascular effects of cocaine.[6]

The N ACC dopamine concentration enhancement in response to cocaine administration was significantly higher in the rats pretreated with alcohol for two weeks. This difference was more significant in the first hour after cocaine administration. Pharmacodynamic analysis showed an approximately 40% increase in the E_{max} for the neurochemical response to cocaine administration. This can be explained by the higher and prolonged cocaine concentrations as a result of alcohol pretreatment. Neurochemical and behavioral sensitization after repeated cocaine treatment has been well documented.[14–16] In our study, however, cocaine pretreatment continued until the day before the challenge dose of cocaine, and it did not have any significant effect on the neurochemical response to an ip cocaine challenge. This lack of neurochemical sensitization may be due to the apparent short term tolerance to the brain ECF dopamine augmentation during the early withdrawal period after repeated cocaine administration.[27]

Dopamine concentration−time profile in response to cocaine administration was significantly higher throughout the experiment period when alcohol was coadministered. The enhanced magnitude and potency of cocaine neurochemical effect was also reflected in the significant changes in the pharmacodynamic model estimates for E_{max} and EC_{50}. This may be explained by the higher cocaine concentrations achieved after combined cocaine and alcohol administration. Because both cocaethylene and norcocaine contribute significantly to the neurochemical response of cocaine,[6,28] the formation of cocaethylene and the increased formation of norcocaine due to alcohol coadministration may further enhance this pharmacological response. These findings imply that, in humans, simultaneous cocaine and alcohol abuse may increase the risk of cocaine-related toxicity in the central nervous system.

Cocaine pretreatment significantly reduced the maximum inhibitory effect of cocaine on the monoamine reuptake, significantly increased the potency of this action, and reduced the maximum mean arterial blood pressure response. These findings indicate that although the maximum increase in mean arterial blood pressure may be lower after repeated exposure to cocaine, the increase in mean blood pressure will be higher for a given cocaine dose after this pretreatment. Alcohol pretreatment increased the potency of the effect of cocaine on the blood pressure. Cocaine and alcohol pretreatments acted together to cause more than an additive effect on increasing the potency of cocaine effect on the mean arterial blood pressure. This may imply that prior use of both cocaine and alcohol can increase the risk of cardiovascular complications in response to cocaine use. Acute alcohol administration with cocaine increased the onset and the dissipation rate constants for the effect of cocaine on the mean arterial blood pressure. This means that alcohol coadministration caused faster increase in the mean arterial blood pressure and faster return to baseline after ip cocaine challenge. The clinical significance of these findings should not be underestimated. Simultaneous administration of cocaine and alcohol may cause higher risks of developing stroke and seizure due to sudden and faster increase in vascular resistance during the first few minutes. Meanwhile, the faster decrease in blood pressure is accompanied by lower vascular tone of the cerebral and epicardial coronary arteries. This may explain the reduced dysphoria such as migraine and chest pain that are often described by abusers of this drug combination as compared to cocaine alone.

The effect of cocaine on heart rate is dependent on its dose. In rats, intra-arterial cocaine doses of up to 0.5 mg/kg increased heart rate, whereas doses above 1.0 mg/kg decreased heart rate.[29] Due to the high cocaine challenge dose used in our study, the heart rate was reduced in all the three groups of rats under investigation. High concentrations of cocaine can block the sodium channel in sensory neurons as well as affect the cardiac action potential leading to slower heart rate and slower cardiac conduction.[30,31] Decreased heart rate may also be caused by activation of vagal baroreceptor reflex.[29] In this study, the time course of the decrease in heart rate in reponse to cocaine administration was not affected by alcohol or cocaine pretreatment. However, pharmacodynamic model parameters showed decreased potency of cocaine effect on the heart rate. On the other hand, alcohol coadministration caused significantly lower heart rate compared to cocaine administration alone, with the most outstanding difference shown in the first hour. Changes in cocaine pharmacokinetics, especially the increase in cocaine AUC_p, the formation of cocaethylene and the increased formation of norcocaine may be responsible for the changes in cocaine pharmacodynamics when alcohol was given with cocaine. Cocaethylene is believed to be implicated in the increased cocaine-related mortality when alcohol is coabused. Cocaethylene has been proven to be more potent than cocaine in mediating lethality in mice.[32] In anaesthetized dogs, cocaethylene causes significant myocardial depression and slight heart rate increase.[33] The reduction in myocardial contractility may lead to a remarkable decrease in hepatic blood flow and, consequently, reduce metabolism of both cocaine, cocaethylene, and norcocaine in the liver. Therefore, changes in the pharmacodynamics with cocaine+alcohol coadministration may in return affect the pharmacokinetics and metabolism of cocaine. However, caution should be exercised when extrapolating these observations in animals to humans. Studies in humans have shown that the appearance of cocaethylene in plasma does not alter subjective and cardiovascular effects of cocaine,[34] and that cocaethylene alone produces milder subjective effects and comparable cardiovascular effects to those of cocaine alone.[35,36]

In conclusion, alcohol pretreatment increases the plasma and brain cocaine concentrations due to inhibition of cocaine elimination. Cocaine pretreatment enhances the systemic bioavailability and clearance of cocaine and increases the formation of norcocaine and cocaethylene. Alcohol coadministration with cocaine increases the plasma and brain cocaine concentrations due to the increase in cocaine systemic bioavailability and reduction of its clearance. Meanwhile, benzoylecgonine formation is significantly reduced, and norcocaine formation is significantly increased. Cocaine and alcohol pretreatments and acute

alcohol coadministration lead to alterations in cocaine pharmacodynamics that are due, at least in part, to the changes in cocaine pharmacokinetics. Alcohol coadministration with cocaine caused significant changes in cocaine neurochemical and cardiovascular responses, and repeated cocaine and alcohol users may respond differently to cocaine administration compared to naïve users. If prior use of alcohol and/or cocaine has similar effects on cocaine pharmacokinetics and pharmacodynamics in humans, the findings of this investigation would indicate that alcoholics and cocaine addicts are at higher risks of cocaine toxicity. The risks may be even higher in these abusers when they consume cocaine and alcohol simultaneously.

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