Cocaine and Alcohol Interactions in the Rat: Contribution of Cocaine Metabolites to the Pharmacological Effects

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Abstract □ The pharmacokinetics and pharmacodynamics of cocaine and its three metabolites, benzoylecgonine, norcocaine, and cocaethylene, were investigated in awake, freely moving rats. This work was performed to examine the effect of alcohol coadministration on the metabolic profile of cocaine and to determine the contribution of cocaine metabolites to the pharmacological responses observed after cocaine administration. The plasma and brain extracellular fluid concentration–time profiles were characterized after intravenous (iv) administration of cocaine and the three metabolites in a crossover experimental design. The neurochemical response, measured as the change in dopamine concentration in the nucleus accumbens, and the cardiovascular responses, measured as the change in the mean arterial blood pressure, heart rate, and QRS interval, were monitored simultaneously. Cocaethylene had the highest brain-to-plasma distribution ratio, followed by cocaine, norcocaine, and benzoylecgonine. The estimated total body clearances for cocaine, benzoylecgonine, norcocaine, and cocaethylene were 140 ± 19, 14.7 ± 1.2, 130 ± 19, and 111 ± 16 mL/min/kg, respectively. Alcohol coadministration increased the formation of norcocaine, decreased the formation of benzoylecgonine, and resulted in the formation of the pharmacologically active metabolite cocaethylene. When cocaine was administered with alcohol, 12.9 ± 3.1% to 15.3 ± 2.9% of the cocaine dose was converted to cocaethylene. Benzoylecgonine did not have any central nervous system or cardiovascular activities after iv administration. Compared with cocaine, norcocaine and cocaethylene had more potent and prolonged effects on the neurochemical, heart rate, and QRS interval responses, and were equipotent in increasing the mean arterial blood pressure. These results indicate that changes in the cocaine metabolic profile and the formation of the pharmacologically active metabolite cocaethylene are, at least partially, responsible for the more intense and longer lasting effects reported after using this drug in combination with alcohol.

Introduction

Cocaine metabolism involves both hydrolysis and oxidation pathways. Benzoylecgonine is formed by spontaneous hydrolysis or as a result of enzymatic hydrolysis by serum and liver microsomal carboxylesterases.[1,2] When both cocaine and alcohol are present, the same enzymes are responsible for the formation of cocaethylene via ethyl transesterification.[2] Ecgonine methyl ester is formed via hydrolysis of the cocaine phenyl ester group by serum and liver cholinesterases.[1,2] Formation of norcocaine is catalyzed by either cytochrome P-450 enzymes or flavin adenine dinucleotide (FAD)-containing monooxygenases.[3−5] Further oxidative metabolism of norcocaine by cytochrome P-450 enzymes yields reactive metabolites that are implicated in the norcocaine-mediated hepatotoxicity of cocaine.[5]

Cocaine is a short-term sympathomimetic psychostimulant that produces marked physiological and behavioral effects in both humans and experimental animals. The pharmacological consequences of cocaine consumption include central nervous system (CNS) stimulant effects manifested by euphoria, increase in locomotor activity, and stereotypy.[6] The cardiovascular activities of cocaine are mainly due to its sympathomimetic effects, which include an increase in blood pressure, QRS duration, and heart rate. However, at higher cocaine concentrations, the sodium channel blocking effect of cocaine can slow the cardiac conduction and result in slower heart rate. The mechanism of the effects of cocaine is believed to be the binding of cocaine to the neurotransmitter reuptake sites, leading to accumulation of the neurotransmitters in the synaptic cleft.[7] The locomotor activity and the reinforcement effects of cocaine are believed to be mediated by the increased brain extracellular (ECF) dopamine concentrations.[8] The major toxicities of cocaine abuse include addiction, cardiac arrhythmia, myocardial ischemia, myocarditis, aortic dissection, cerebral vasoconstriction, seizure, and trauma that leads to death.[9] The proposed mechanism for the cardiac arrhythmia and conduction disturbances associated with cocaine overdose is the blockage of cardiac sodium channels by cocaine.[10,11]

The two major metabolites of cocaine, benzoylecgonine and ecgonine methyl ester, do not have any cocaine-like stimulant activity when administered to experimental animals. However continuous intravenous (iv) infusion of benzoylecgonine and ecgonine methyl ester in doses of 0.45 and 1.5 mg/kg/min, respectively, for 30 min to anesthetized rats have been shown to significantly increase the blood pressure without affecting either the heart rate or the QRS duration.[11] Norcocaine can cause a CNS stimulant effect in rats after intracerebroventricular administration and has higher affinity for inactivated cardiac sodium channels than cocaine in guinea pig cardiac myocytes.[10,12] Norcocaine is also hepatotoxic in both animals and humans.[5,13] Cocaethylene is a CNS stimulant and has cardiovascular effects comparable to those of cocaine in rats and rabbits.[11,14]

Alcohol coadministration with cocaine has been shown to alter the metabolic profile of cocaine, leading to the formation of cocaethylene and increasing the fraction of cocaine dose converted to norcocaine.[15] The changes in cocaine pharmacokinetics and metabolic profile has been implicated in the more intense and longer-lasting cocaine pharmacological effects observed after abusing this drug combination.[16] The primary objective of this study was to investigate the pharmacokinetics and pharmacodynamics of cocaine and its three metabolites, benzoylecgonine,

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norcocaine, and cocaethylene, in the rat. This work was important to determine the effect of alcohol coadministration on the metabolic profile of cocaine and to evaluate the contribution of cocaine metabolites to the pharmacological effects of cocaine. In this study, the pharmacokinetics of cocaine and its metabolites were characterized after iv administration of the four compounds to the rat in a crossover experimental design. Meanwhile, the neurochemical response, measured as the change in dopamine concentration in the nucleus accumbens (N ACC), and the cardiovascular responses, measured as changes in mean arterial blood pressure, heart rate, and QRS interval, were monitored simultaneously after each drug administration. This is the first study that examined the pharmacokinetics and pharmacodynamics of cocaine metabolites and compared the effect of cocaine and its metabolites on the pharmacological activities in the same group of experimental animals.

Materials and Methods

Chemicals and Reagents—Benzoylecgonine, norcocaine hydrochloride, and cocaethylene hydrochloride were obtained from Research Biochemicals International (Natick, MA). Cocaine hydrochloride was purchased from Sigma Chemical (St. Louis, MO).

Animal Care and Preparation—Male Wistar rats (250−350 g, Simonsen Laboratories, Gilroy, CA) were used in this investigation. 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. The details of the animal preparation procedures were described previously.[17,18]

Administration of Cocaine and its Metabolites—Stock solutions of cocaine (10 mg/mL), benzoylecgonine (5 mg/mL), norcocaine (1.5 mg/mL), and cocaethylene (3 mg/mL) in normal saline were prepared and used for the experiment. Drugs were administered iv through the femoral vein cannula at doses of 0.02 mmol/kg (for cocaine) and 0.01 mmol/kg (for benzoylecgonine, norcocaine, and cocaethylene) in a crossover experimental design. The cocaine dose was chosen because it is well below the dose of cocaine that is lethal in 50% of the tested rats (LD_{50}) after iv administration (0.05 mmol/kg, Material Safety Data Sheet, University of Washington, Seattle, 1995). The dose of 0.01 mmol/kg for benzoylecgonine, norcocaine, and cocaethylene was chosen based on the preliminary studies conducted in our laboratory to determine the tolerable doses of these metabolites in the rat. The doses of cocaine and its metabolites used in this experiment should achieve measurable plasma and brain concentrations of these compounds for at least 2 h after drug administration.

Pharmacokinetic and Pharmacodynamic Studies—Nine male Wistar rats were randomly chosen and were prepared following the surgical procedures described previously.[17,18] After the recovery period, the rats were treated with cocaine, benzoylecgonine, norcocaine, and cocaethylene in a crossover experimental design, with a 24-h washout period between treatments.

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 three exposed tips of the insulated wires of the 3-electrode EKG cable were connected to the sinus rhythm analyzer (Digi-Med model 200, Micro-Med, Louisville, KY) in Lead-II setting for monitoring the cardiac electrical activity. The signals from the two analyzers were collected, updated, and averaged every 1 min 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) in HPLC autosampler vials containing 20 μL of dopamine mobile phase. After mixing the vial content, 5 μL was injected immediately into an HPLC equipped with an electrochemical (EC) detector for dopamine analysis. This procedure was repeated until a stable basal dopamine concentration was detected (<10% difference in dopamine concentration in three consecutive samples). Once a stable dopamine baseline was achieved, the rat received iv cocaine (or one of the three metabolites) through the femoral vein cannula. 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, and 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 analysis 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 in HPLC autosampler vials containing 20 μL of dopamine mobile phase (pH 4). This procedure was to maintain dopamine, cocaine, and its metabolites under acidic condition so as 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 remainder was analyzed for cocaine and its metabolites by HPLC−UV. The mean arterial blood pressure, the heart rate, and the QRS interval were continuously monitored during the entire experiment. Nine rats received all three metabolites in a crossover experimental design, whereas only six of these rats received cocaine in addition to the three metabolites.

The pharmacokinetic parameters for each of the cocaine metabolites estimated in the current study were utilized to investigate the effect of alcohol on cocaine metabolic profile. This was achieved by estimating the fraction of the cocaine dose converted to each of the metabolites when cocaine was administered alone and in combination with alcohol. The results obtained from a previous pharmacokinetic experiment, which involved iv and intraperitoneal (ip) cocaine administrations, were used in this analysis.[18] The pharmacological effects observed after the administration of benzoylecgonine, norcocaine, and cocaethylene were used to determine the contribution of these metabolites to the overall cocaine pharmacological effects when cocaine was administered alone and in combination with alcohol.

Analytical Methods—*Cocaine and Its Metabolites*—Plasma and microdialysis probe effluents were analyzed for cocaine and its metabolites by the method developed in our laboratory.[19] This method is sensitive enough to quantitate cocaine and its metabolites in concentrations as low as 0.075 nmol/mL in 100-μL plasma samples, with coefficient of variation of <10%. The brain ECF concentrations of cocaine and its metabolites were determined by the same method, except that the probe effluent was injected directly into the HPLC−UV system without any pretreatment. 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.

Dopamine—The microdialysis probe effluent was injected directly into an HPLC system equipped with an EC detector for dopamine analysis immediately after collection. The details of the analytical procedures used for dopamine determination in the microdialysis probe effluent were described previously.[18]

Pharmacokinetic Analysis—A two-compartment pharmacokinetic model with elimination from the central compartment was used to analyze the distribution and elimination of cocaine and its metabolites after iv administration.[18] This model assumes that the distribution and elimination of these compounds follow first-order kinetics, and that the brain is part of the peripheral tissue compartment. The plasma and brain ECF drug concentrations for each rat after administrations of each compound were fitted simultaneously to the equations that describe the plasma and brain ECF concentration−time profiles.[18] The pharmacokinetic model parameters were estimated by nonlinear regression analysis utilizing PCNONLIN (Version 4.0, SCI Software, Lexington, KY). Other pharmacokinetic parameters such as the total body clearance (TBC), the volumes of distribution at steady state and during the elimination phase (Vd_{ss} and Vd_{β}), and the area under the drug plasma (AUC_p) and brain ECF (AUC_b) concentration−time curves were calculated from the estimated parameters.[20]

The fraction of the cocaine iv dose converted to each metabolite (f_m) was calculated using eq 1:[21]

$$f_m = \frac{AUC_{\text{iv cocaine}}(m)TBC(m)}{D_{iv}} \quad (1)$$

where $AUC_{\text{iv cocaine}}(m)$ is the area under the plasma metabolite

concentration−time curve after iv administration of cocaine, D_{iv}, is cocaine dose, and TBC(m) is the total body clearance of the metabolite after iv administration of cocaine, which is assumed to be equal to the estimated metabolite TBC after administration of the preformed metabolite. For ip cocaine administration, the fraction of the cocaine dose converted to the metabolite (f_m) was also calculated using eq 1 after substituting $AUC_{iv\ cocaine}(m)$ and D_{iv} by $AUC_{ip\ cocaine}(m)$ and D_{ip}.

Pharmacodynamic Analysis—*Neurochemical Response*—The linear pharmacodynamic model was used to describe the relationship between the brain ECF drug concentration and the change in the brain ECF dopamine concentration. The mathematical expression that describes the concentration−effect relationship for the model is the following:

$$E = E_o + \text{slope}\ C_b \tag{2}$$

where E is the effect measured as the percent change in dopamine concentration, E_0 is the baseline effect measured as the basal dopamine concentration, Slope is the percent increase in the measured dopamine concentration caused by every unit increase in the brain ECF drug concentration (C_b). In this model, the slope is a measure of the potency of the drug. The pharmacodynamic model parameters were estimated by fitting the percent change in dopamine level and the drug concentration in the brain ECF at different time points to eq 2. The basal dopamine concentration was kept constant (100%) during the linear regression analysis.

Cardiovascular Responses—The relationship between the plasma drug concentration and the change in the mean arterial blood pressure, heart rate, and QRS interval after iv administration of the four compounds was characterized by the mechanism-based pharmacodynamic response model as described previously.[22,23] The mathematical expression that describes the model for the change in the pharmacological response and the drug concentration is the following:

$$\frac{dR}{dt} = k_{in} - k_{out}\left(1 - \frac{I_{max}C_p^{\,n}}{IC_{50}^{\,n} + C_p^{\,n}}\right)R \tag{3}$$

where R is the observed response (for example the percent change in mean arterial blood pressure), 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 drug concentration that leads to 50% inhibition of the factor that produces the effect, C_p is the plasma drug concentration at the time of the observed response, and n is the sigmoidicity factor. At very high drug concentration or at the maximum inhibition, the maximum response can be estimated as follows[22,23]

$$R_{max} = \frac{R_0}{1 - I_{max}} \tag{4}$$

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

The pharmacodynamic model parameters were estimated by fitting the percent change in each of the monitored cardiovascular functions and the plasma concentrations of cocaine and its metabolites at different time points to eq 3. Nonlinear regression analysis was performed utilizing PCNONLIN.

Statistical Analysis—Cocaine and its metabolites were administered to the rats in a crossover experimental design. The estimated pharmacodynamic parameters for the neurochemical and the cardiovascular effects of each compound were compared by the paired t test. Also, the effect of alcohol coadministration with cocaine on the pharmacokinetic parameters of each metabolite were compared by the paired t test. Differences of $p < 0.05$ were considered significant.

Results

The plasma and the brain concentration−time profiles of cocaine, benzoylecgonine, norcocaine, and cocaethylene after a single iv bolus administration are illustrated in Figure 1. For these four compounds, the plasma concentration declined biexponentially and the brain ECF concentration increased rapidly and then declined parallel to the concentration in plasma during the elimination phase. The distribution half-lives of cocaine and its metabolites were not significantly different from each other and they ranged from 4.48 ± 0.51 to 6.4 ± 1.1 min. On the other hand, benzoylecgonine had the longest elimination half-life, followed by norcocaine, cocaethylene, and cocaine. Benzoylecgonine had the smallest TBC, followed by cocaethylene, norcocaine, and then cocaine. The brain-to-plasma distribution ratio, measured as AUC_b/AUC_p, was the highest for cocaethylene, followed by cocaine, norcocaine, and benzoylecgonine. A summary of the model-estimated pharmacokinetic parameters of cocaine, benzoylecgonine, norcocaine, and cocaethylene is listed in Table 1.

After iv administration of cocaine, norcocaine, and cocaethylene, the brain ECF dopamine concentration increased and reached its maximum value within 20−40 min, then it gradually declined to its baseline value after 2 h when the brain ECF drug concentration approached zero. The dopamine concentration−time profile followed the drug concentration−time profile in the N ACC. Benzoylecgonine, however, did not cause any significant change in N ACC dopamine concentration (Figure 2A). The relationship between the brain ECF drug concentration and the percent change in dopamine concentration was determined with the linear pharmacodynamic model. Representative examples of the linear model-fitted lines in one rat after iv administration of cocaine, norcocaine, and cocaethylene are shown in Figure 2B. Norcocaine and cocaethylene were more potent than cocaine with respect to the neurochemical effect, and the average model-estimated slopes for these two compounds were 3.7 and 2.5 times larger than that of cocaine (Table 2).

The mean arterial blood pressure increased rapidly after cocaine administration and then declined slowly and did not return to its baseline value when plasma drug concentration approached zero (Figure 3A). The effect of cocaine on the mean arterial blood pressure was higher than that of norcocaine and cocaethylene, however, this difference was due to the higher cocaine dose. The pharmacodynamic analysis showed that there was no difference in the estimated IC_{50} and R_{max} for the effect of cocaine, norcocaine, and cocaethylene on the mean arterial blood pressure, which indicates that there is no differences in the potency and the maximum intrinsic activity of these three compounds (Table 2). Norcocaine and cocaethylene caused larger reductions in heart rate even though their doses were 50% lower than that of cocaine (Figure 3B). The higher potency of these two cocaine metabolites was reflected in their significantly lower IC_{50} compared with that of cocaine (Table 2). For the QRS response, norcocaine and cocaethylene were more potent than cocaine, as indicated by their lower IC_{50} (Table 2). Benzoylecgonine did not have any significant effect on the mean arterial blood pressure, heart rate, or QRS interval (Figures 3A−C). Representative examples of the observed cardiovascular response values and the indirect pharmacodynamic response model-fitted curves for one rat after administration of cocaine and its metabolites in a crossover experimental design are shown in Figures 4A−C. All estimated parameters of cocaine and its metabolites for the pharmacodynamic model are summarized in Table 2.

In one of our previous cocaine pharmacokinetic studies, we administered cocaine iv and ip to rats with and without alcohol.[18] The plasma and brain ECF concentration−time profiles of cocaine and its metabolites after ip cocaine with and without the coadministration of alcohol are shown in Figure 5. After ip cocaine alone, both benzoylecgonine and norcocaine were detected in the plasma and the brain (Figures 5A & B). In the presence of alcohol, the pharma-

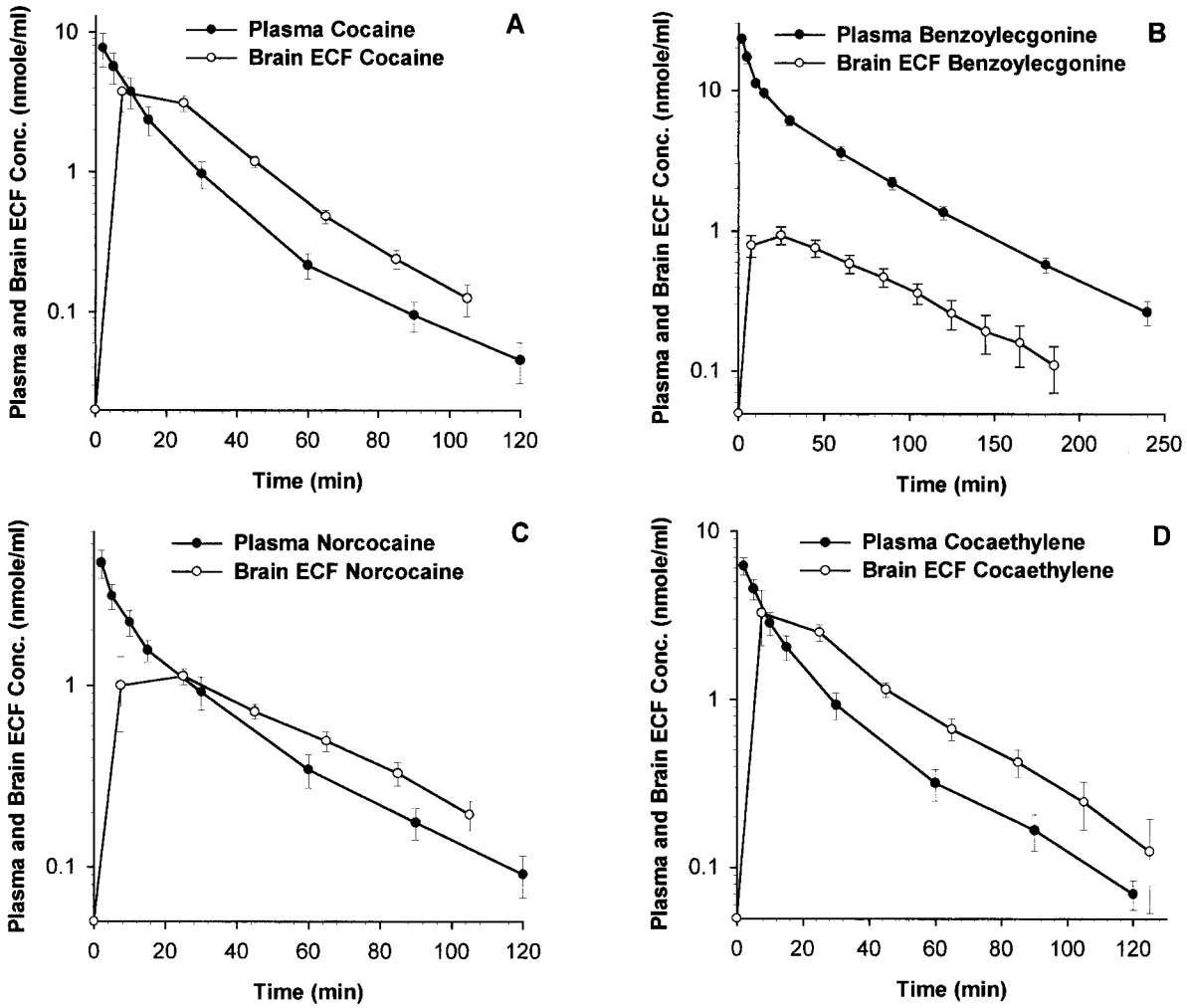

Figure 1—Plasma (●) and brain ECF (○) concentration–time profiles of cocaine and its metabolites after administration of 0.02 mmol/kg cocaine iv (A), 0.01 mmol/kg benzoylecgonine iv (B), 0.01 mmol/kg norcocaine iv (C), and 0.01 mmol/kg cocaethylene iv (D) to the rat. (Data presented as mean ± SE; $n = 6$ for cocaine and $n = 9$ for the metabolites.)

Table 1—Pharmacokinetic Parameters of Cocaine, Benzoylecgonine, Norcocaine, and Cocaethylene after iv Administration of 0.02 mmol/kg of cocaine and 0.01 mmol/kg of Each of the Cocaine Metabolites to the Rat[a]

	administered compound			
pharmacokinetic parameter	cocaine	benzoylecgonine	norcocaine	cocaethylene
AUC_p (nmol·min/mL)	157 ± 19	720 ± 55	93 ± 15	108 ± 16
AUC_b (nmol·min/mL)	170 ± 23	94 ± 13	78.5 ± 9.6	149 ± 26
AUC_b/AUC_p	1.18 ± 0.26	0.147 ± 0.032	0.97 ± 0.14	1.44 ± 0.17
TBC (mL/min/kg)	140 ± 19	14.7 ± 1.2	130 ± 19	111 ± 16
$\alpha_{t1/2}$ (min)	6.2 ± 1.3	4.48 ± 0.51	6.4 ± 1.1	5.35 ± 0.97
$\beta_{t1/2}$ (min)	15.8 ± 1.5	46.7 ± 2.9	38.3 ± 5.1	25.0 ± 3.1
k_{12} (min^{-1})	0.025 ± 0.014	0.081 ± 0.013	0.072 ± 0.033	0.097 ± 0.052
k_{21} (min^{-1})	0.085 ± 0.018	0.070 ± 0.010	0.087 ± 0.047	0.093 ± 0.033
k_{10} (min^{-1})	0.076 ± 0.007	0.041 ± 0.002	0.059 ± 0.004	0.080 ± 0.006
V_c (L/kg)	1.96 ± 0.34	0.364 ± 0.033	2.22 ± 0.30	1.43 ± 0.20
Vd_{ss} (L/kg)	2.48 ± 0.60	0.808 ± 0.065	4.06 ± 0.52	2.34 ± 0.31
Vd_β (L/kg)	3.28 ± 0.64	1.00 ± 0.12	6.54 ± 0.84	4.14 ± 0.88

[a] Values are presented as mean ± SE; $n = 6$ for cocaine; $n = 9$ for metabolites.

cologically active metabolite cocaethylene was formed and it was detected in both plasma and the brain (Figures 5C & D). The effect of alcohol coadministration on the metabolic profile of cocaine was determined utilizing the pharmacokinetic parameters of cocaine metabolites determined in the current study, and the results are summarized in Table 3.

Compared with iv cocaine alone, alcohol coadministration caused significant inhibition of cocaine metabolism to benzoylecgonine. This effect is shown in the significant reduction in benzoylecgonine AUC_p and $C_{p\ max}$. Also, the fraction of the administered cocaine dose converted to benzoylecgonine was significantly reduced from 0.432 ± 0.047 to 0.237 ± 0.038, and the formation clearance of this cocaine metabolite was decreased from 47.2 ± 4.0 to 27.2 ± 4.0 mL/min/kg. Alcohol coadministration also led to a slight increase in the formation of norcocaine, and cocaethylene was formed only when alcohol was administered

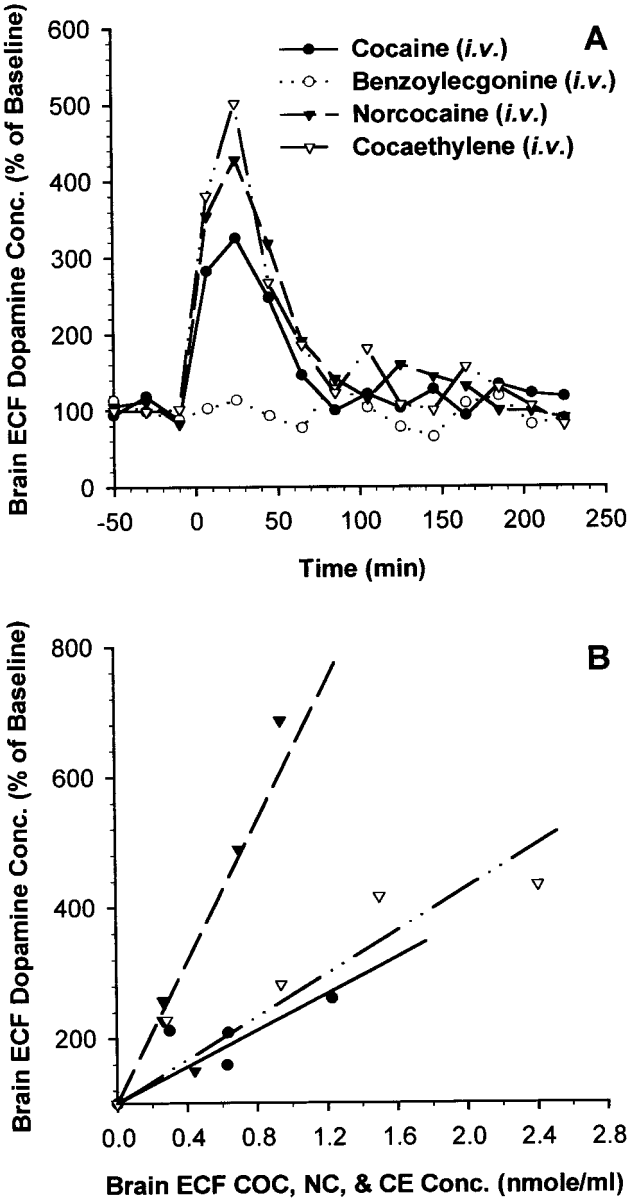

Figure 2—(A) The brain ECF dopamine concentration–time profiles after administration of 0.02 mmol/kg cocaine iv (●), 0.01 mmol/kg benzoylecgonine iv (○), 0.01 mmol/kg norcocaine iv (▼), and 0.01 mmol/kg cocaethylene iv (▽) to the rat. Each point represents the mean from six rats. (B) Representative examples of the relationship between the percent change in the brain ECF dopamine concentrations and cocaine (●), norcocaine (▼), and cocaethylene (▽) brain ECF concentrations after iv administration of these compounds in one rat in a crossover experimental design. The symbols represent the observed values and the lines represent the linear model-fitted curves.

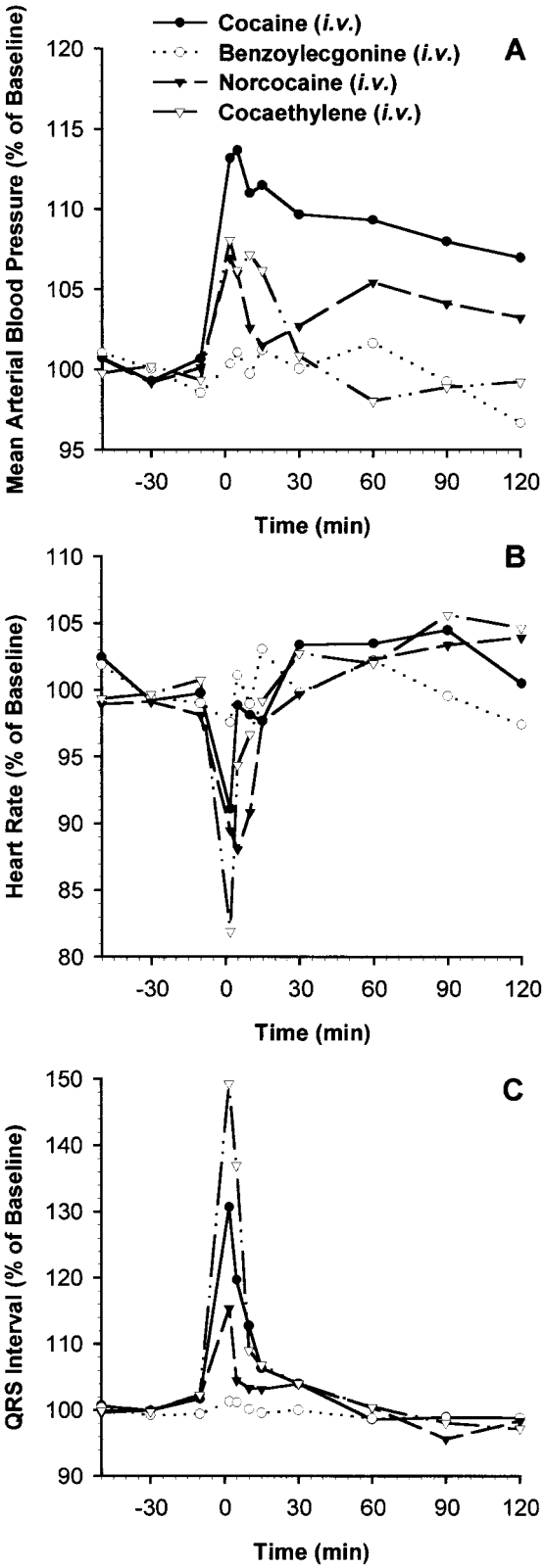

Figure 3—Percent change in the mean arterial blood pressure (A), heart rate (B), and QRS interval (C) after administration of 0.02 mmol/kg cocaine iv (●), 0.01 mmol/kg benzoylecgonine iv (○), 0.01 mmol/kg norcocaine iv (▼), and 0.01 mmol/kg cocaethylene iv (▽) to the rat. Each point represents the mean from six rats.

with cocaine. The fraction of cocaine iv dose converted to cocaethylene was 0.129 ± 0.031. When cocaine was given ip, alcohol coadministration caused significant reduction in benzoylecgonine $C_{p\ max}$. However, no changes were found in the fraction of the cocaine dose converted to benzoylecgonine. There was about a 40% increase in the formation of norcocaine, and 15.3 ± 2.9% of the cocaine dose was metabolized to cocaethylene.

Discussion

In the present study, we investigated the pharmacokinetics and pharmacodynamics of cocaine and its three metabolites simultaneously after iv bolus administration of these compounds to awake, freely moving rats. Our results showed that the highly hydrophilic cocaine metabolite benzoylecgonine had the highest AUC_p, whereas its AUC_b was the lowest. The brain-to-plasma distribution ratio of benzoylecgonine was the lowest among the four

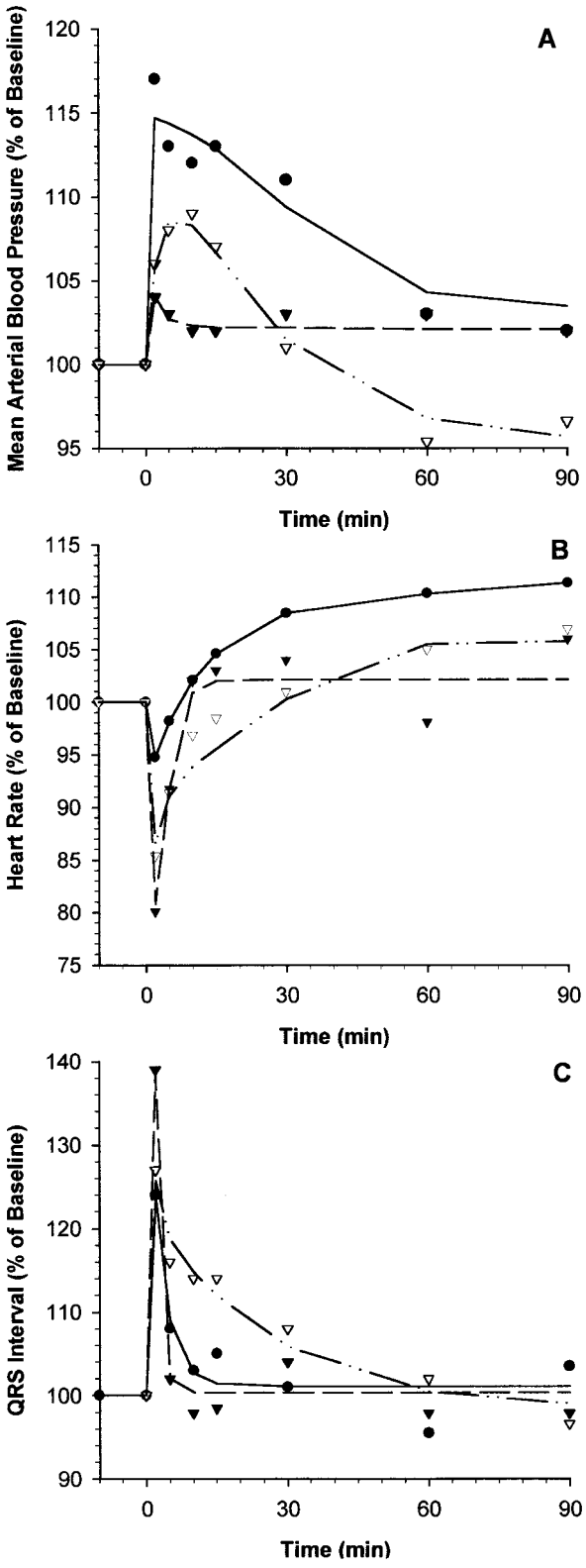

Figure 4—Representative examples of the percent change in the time profiles of the mean arterial blood pressure (A), heart rate (B), and QRS interval (C) after administration of 0.02 mmol/kg cocaine iv (●), 0.01 mmol/kg norcocaine iv (▼), and 0.01 mmol/kg cocaethylene iv (▽) to one rat in a crossover experimental design. The symbols represent the observed values and the lines represent the pharmacodynamic model-fitted curves.

compounds under investigation. Benzoylecgonine iv administration did not have any effect on dopamine levels in the N ACC or on the monitored cardiovascular functions. The absence of the CNS effect after an iv bolus dose of

Table 2—Pharmacodynamic Parameters after iv Administration of Cocaine (0.02 mmol/kg) and Its Metabolites (0.01 mmol/kg) to the Rat (Mean ± SE)

pharmacodynamic parameter	compound		
	cocaine	norcocaine	cocaethylene
	A. neurochemical		
Slope (%/nmol/mL)	85 ± 18	314 ± 97[b]	211 ± 73
	B. cardiovascular		
	mean blood pressure		
k_{out} (min^{-1})	1.38 ± 0.86	1.95 ± 0.93	1.72 ± 0.80
I_{max}	0.277 ± 0.087	0.231 ± 0.051	0.239 ± 0.094
IC_{50} (nmol/mL)	8.4 ± 3.1	7.2 ± 3.4	4.7 ± 2.4
R_{max} (% of baseline)	152 ± 22	133 ± 8.6	146 ± 24
	heart rate		
k_{out} (min^{-1})	3.02 ± 0.77	1.92 ± 0.75	3.44 ± 0.36
I_{max}[a]	0.359 ± 0.072	0.273 ± 0.075	0.45 ± 0.11[c]
IC_{50} (nmol/mL)	12.5 ± 4.7	1.83 ± 0.47[b]	6.6 ± 1.4[c]
R_{max} (% of baseline)[a]	165 ± 16	147 ± 18	248 ± 69[c]
	QRS interval		
k_{out} (min^{-1})	5.31 ± 0.45	0.94 ± 0.20[b]	2.91 ± 0.98[c]
I_{max}	0.63 ± 0.18	0.26 ± 0.14	0.47 ± 0.13
IC_{50} (nmol/mL)	14.4 ± 5.0	3.4 ± 1.5	5.8 ± 1.7
R_{max} (% of baseline)	490 ± 260	149 ± 35	227 ± 74

[a] Presented as absolute values. [b] Significantly different from the cocaine treatment ($p < 0.05$, paired t test). [c] Significantly different from the norcocaine treatment ($p < 0.05$, paired t test).

benzoylecgonine may be explained by the low brain distribution of this hydrophilic metabolite because intracerebroventricular administration of benzoylecgonine has been shown to produce stimulant effect in awake Sprague-Dawley rats.[12] A previous investigation has shown that benzoylecgonine can increase the blood pressure after iv infusion of 0.45 mg/kg/min for 30 min to anesthetized rats; however, the dose of benzoylecgonine used in that investigation was much higher than the dose used in our experiment.[11]

Cocaethylene has the highest brain-to-plasma distribution ratio, followed by cocaine, norcocaine, and then benzoylecgonine, which is the same rank order of the lipophilicity of these four compounds. This result indicates that the brain distribution of these compounds is probably via passive diffusion across the blood–brain barrier. Norcocaine and cocaethylene administration resulted in higher dopamine concentration in the N ACC compared with after cocaine administration. The brain ECF concentration–neurochemical effect relationships for cocaine, norcocaine, and cocaethylene after iv administration were best described by the linear pharmacodynamic model, even though we used the sigmoid-E_{max} pharmacodynamic model to describe the brain ECF concentration–neurochemical effect relationship after ip cocaine administration.[17] The reason for this discrepancy is that the iv dose used in the current investigation was much lower than the ip dose that resulted in lower brain ECF concentration of cocaine and its metabolites. It was not possible to characterize the sigmoid-E_{max} relationship because all the observed brain ECF concentrations were in the lower linear portion of this relationship. The linear pharmacodynamic model showed that norcocaine and cocaethylene were more potent than cocaine in dopamine reuptake inhibition. This result was consistent with the higher dopamine concentrations observed after norcocaine and cocaethylene administration despite the fact that cocaine dose was twice that of norcocaine and cocaethylene. This finding indicates that after cocaine administration with alcohol, despite the lower concentrations of norcocaine and cocaethylene, these metabolites may be significantly contributing to the observed neurochemical response to cocaine administration due to

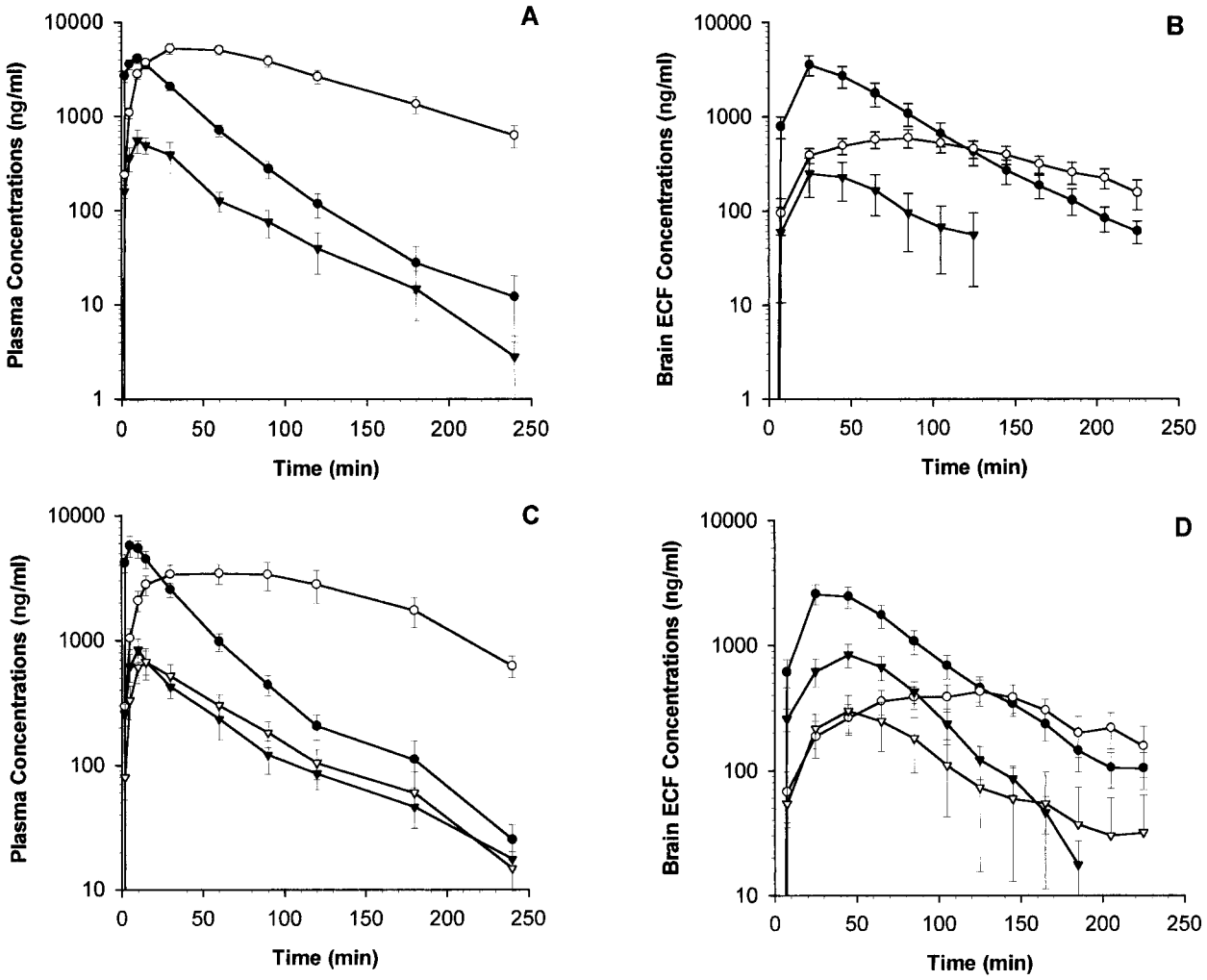

Figure 5—Plasma (A, C) and the brain (B, D) ECF concentration–time profiles of cocaine (●), benzoylecgonine (○), norcocaine (▼), and cocaethylene (▽) after administration of 0.088 mmol/kg cocaine ip (A, B) and 0.088 mmol/kg cocaine ip plus 5 g/kg alcohol po (C, D) to the rat in a balanced crossover experimental design. (Data presented as mean ± SE, $n = 8$.)

Table 3—Cocaine Metabolite Pharmacokinetics after Cocaine iv and ip Administrations Alone or In Combination with Alcohol (Mean ± SE)

pharmacokinetic parameter	cocaine (iv) + NS (po)[a]	cocaine (iv) + alcohol (po)[a]	cocaine (ip) + NS (po)[b]	cocaine (ip) + alcohol (po)[b]
		cocaine		
AUC_p (nmol·min/mL)	187 ± 17	174 ± 114	453 ± 35	644 ± 100[c]
$C_{p\,max}$ (nmol/mL)	10.9 ± 1.1	11.0 ± 0.7	12.1 ± 1.4	17.4 ± 3.1[c]
TBC (mL/min/kg)	118 ± 12	125 ± 12	112 ± 4	106 ± 5
		benzoylecgonine		
AUC_p (nmol·min/mL)	587 ± 64	420 ± 130[c]	2000 ± 210	2020 ± 430
$C_{p\,max}$ (nmol/mL)	4.33 ± 0.50	1.59 ± 0.27[c]	15.0 ± 1.8	10.8 ± 2.3[c]
f_m	0.432 ± 0.047	0.237 ± 0.038[c]	0.326 ± 0.036	0.336 ± 0.071
f_m TBC (mL/min/kg)	47.2 ± 4.0	27.2 ± 4.0[c]	34.9 ± 3.8	33.3 ± 4.3
		norcocaine		
AUC_p (nmol·min/mL)	2.3 ± 1.2	3.0 ± 1.1	79 ± 19	110 ± 23
$C_{p\,max}$ (nmol/mL)	0.155 ± 0.029	0.198 ± 0.044	1.64 ± 0.43	2.41 ± 0.51[c]
f_m	0.015 ± 0.008	0.017 ± 0.007	0.116 ± 0.028	0.162 ± 0.033
f_m *TBC* (mL/min/kg)	1.46 ± 0.74	2.01 ± 0.86	12.4 ± 3.0	16.3 ± 2.7
		cocaethylene		
AUC_p (nmol·min/mL)		23.2 ± 5.6		122 ± 23
$C_{p\,max}$ (nmol/mL)		0.259 ± 0.043		1.78 ± 0.48
f_m		0.129 ± 0.031		0.153 ± 0.029
f_m TBC (mL/min/kg)		16.2 ± 4.4		15.3 ± 1.9

[a] Balanced crossover experiment, $n = 12$; cocaine dose, 0.02 mmol/kg; alcohol dose, 5 g/kg. [b] Balanced crossover experiment, $n = 8$; cocaine dose, 0.088 mmol/kg; alcohol dose, 5 g/kg. [c] Significantly different from cocaine + normal saline ($p < 0.05$, paired t test).

their higher potency. Also, factors that can alter the formation of these two metabolites, such as genetic polymorphism and drug-drug interactions, may change the overall neurochemical response to cocaine administration.

Analysis of the cardiovascular effects observed after administration of cocaine and its metabolites showed that cocaine, norcocaine, and cocaethylene have similar potency and maximum intrinsic activity on the mean arterial blood pressure. Both norcocaine and cocaethylene were more potent than cocaine in reducing the heart rate and prolonging the QRS interval. This result is consistent with the previous experimental results obtained in vitro using cardiac myocytes.[10,24] Meanwhile, k_{out} was the smallest for norcocaine, so that the dissipation of the heart rate response was the slowest when rats were treated with norcocaine. Norcocaine and cocaethylene also had smaller k_{out} values compared with cocaine, which means slower dissipation of the QRS response after administration of these two compounds. These pharmacodynamic results were consistent with the pharmacokinetic findings because norcocaine and cocaethylene have longer elimination half-lives and lower TBCs when compared with cocaine. The pharmacodynamic model-predicted higher potency of cocaethylene and norcocaine with respect to their effect on heart rate is consistent with the higher responses observed after administration of these two metabolites. These findings indicate that norcocaine and cocaethylene should be contributing to the cardiovascular responses to cocaine administration because of their higher potency.

The fraction of the cocaine iv dose converted to benzoylecgonine was reduced by >50% when iv cocaine was administered with alcohol. However, the formation of benzoylecgonine was not reduced after ip administration of cocaine with alcohol. The difference in the fraction of the iv and the ip doses of cocaine metabolized to benzoylecgonine (0.43 for iv versus 0.33 for ip) indicates that less benzoylecgonine is formed during the presystemic metabolism of cocaine compared with the systemic metabolism of cocaine. Coadministration of alcohol results in increased cocaine bioavailability after ip administration, which will lead to the formation of more benzoylecgonine and may mask the effect of alcohol coadministration on benzoylecgonine formation. This result may explain the small difference in the fraction of cocaine dose metabolized to benzoylecgonine after cocaine ip alone and after cocaine ip and alcohol.

Norcocaine formation after ip cocaine administration was ≈8 times more than that after iv cocaine administration. This difference may be caused by more norcocaine formation during the presystemic metabolism when cocaine was given ip. The reason for this result is that the formation of norcocaine is catalyzed by either cytochrome P-450 enzymes (CYP2B and CYP3A subfamilies) or FAD-containing monooxygenase.[3] These enzymes, especially the CYP3A subfamily, have different expressions and activities in the gut and liver isoforms.[25] Norcocaine formation increased by 10 and 40% due to alcohol coadministration with iv and ip cocaine administration, respectively. Our pharmacodynamic analysis showed that norcocaine is more potent than cocaine in its neurochemical, heart rate, and QRS interval effects. Norcocaine AUC in plasma was ≈1−2% as large as cocaine AUC after iv cocaine administration and was ≈17% as large as cocaine AUC after ip cocaine administration. Because of the higher potency of norcocaine this metabolite significantly contributes to the observed neurochamical and cardiovascular effects after ip cocaine administration because 11−16% of cocaine dose is converted to norcocaine. However, after iv cocaine administration, which is the route of administration relevent to human cocaine abuse, the contribution of norcocaine to the neurochamical and cardiovascular effects may not be significant because only 1−2% of the administered iv cocaine dose is converted to norcocaine.

The hepatotoxicity of norcocaine is believed to be the result of its further oxidative metabolism by CYP2Bs, either through redox cycling between *N*-hydroxynorcocaine and norcocaine nitroxide or the production of an unidentified reactive metabolite.[5,26] The hepatic toxicity of norcocaine may be synergized by alcohol because both of these drugs act on the central lobular area of the liver.[27] In addition to potentiation of cocaine-induced hepatocellular toxicity through CYP2E1-dependent oxidative stress, alcohol could also enhance cocaine bioactivation through induction of other P-450 enzymes, such as the CYP2B and 3A subfamilies.[26,28] In our investigation, the increase in norcocaine formation due to a single dose of alcohol was ≈10% after iv cocaine administration and 40% after ip cocaine administration. This difference may indicate that the increase in cocaine hepatotoxicity when given in combination with alcohol is not exclusively dependent on the increased norcocaine formation especially after iv cocaine administration.

Approximately 13 to 15% of the cocaine doses were converted to cocaethylene when cocaine was administered iv or ip in combination with alcohol. Presystemic and systemic metabolism led to similar fractional conversion of cocaine to cocaethylene. Our pharmacodynamic analysis showed that cocaethylene is ≈2−3 times more potent than cocaine in its neurochemical, heart rate, and QRS interval effects. The pharmacokinetic analysis showed that cocaethylene AUC in plasma and brain were ≈15−20% as large as those of cocaine after administration of cocaine and alcohol. Because of its higher potency, cocaethylene should be significantly contributing to the pharmacological effects observed in response to combined cocaine and alcohol administration even if cocaethylene concentration in brain and plasma is much lower than that of cocaine.

In conclusion, benzoylecgonine does not possess any significant CNS or cardiovascular activities after iv administration with the dose used in our investigation. Norcocaine and cocaethylene are more potent than cocaine with respect to their neurochemical, heart rate, and QRS interval effects. However, cocaine, norcocaine, and cocaethylene are equipotent with respect to their effect on the mean arterial blood pressure. Alcohol coadministration causes the formation of the pharmacologically active metabolite cocaethylene, reduces the formation of the inactive metabolite benzoylecgonine, and increases the formation of norcocaine. Because of its higher potency, cocaethylene should have significant contribution to the observed pharmacological effects after administration of cocaine and alcohol. Also, modification of the cocaine metabolic profile, such as that due to drug−drug interactions, can lead to significant modification of the observed cocaine pharmacological effects. Because our investigation has been performed in rodents and differences in cocaine pharmacokinetics and pharmacodynamics in different species have been reported, the clinical significance of these finding warrants further investigation.

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