Anesthesiology
1999; 90:1684–90

The Comparative Toxicity of Cocaine and Its Metabolites in Conscious Rats

Hisayo O. Morishima, M.D., Ph.D., Robert A. Whittington, M.D.,† Ayako Iso, M.D.,‡ Thomas B. Cooper, M.A.§*

Background: **The metabolites of cocaine, benzoylecgonine and ecgonine methyl ester, have been considered pharmacologically inactive when administered systemically. However, recent *in vitro* studies suggest that this may not be true. The current study was designed to evaluate the systemic toxicity of cocaine and its metabolites when administered systemically to awake rats fitted with catheters for long-term monitoring.**

Methods: **Cocaine, norcocaine, cocaethylene, benzoylecgonine, and ecgonine methyl ester were infused intravenously to produce sequential behavioral alterations and central nervous system and cardiovascular toxic effects. Arterial blood pressure and heart rate were monitored continuously. Plasma and tissue samples were analyzed for all compounds by capillary gas chromatography–mass spectrometry.**

Results: **The dose of norcocaine necessary to produce toxic effects was smaller than that of cocaine and cocaethylene. Benzoylecgonine and ecgonine methyl ester did not produce toxic manifestations at infusion rates that produced toxicity in the cocaine, norcocaine, and cocaethylene groups. Furthermore, 30- and 60-fold higher doses of benzoylecgonine and ecgonine methyl ester, respectively, were necessary to produce only mild neurobehavioral changes. Benzoylecgonine was not lethal even at doses 100 times greater than cocaine.**

Conclusions: **These results indicate that benzoylecgonine and ecgonine methyl ester are not as toxic as cocaine, norcocaine, or cocaethylene when administered intravenously to pharmacologically naive rats. (Key words: Cocaine; metabolites; toxicity.)**

COCAINE is metabolized in mammals mainly in the liver and the plasma and depends on two metabolic pathways: deesterification and N-demethylation.[1] Deesterification results in the formation of the major metabolites, benzoylecgonine and ecgonine methyl ester,[2,3] whereas N-demethylation results in a pharmacologically active metabolite, norcocaine.[4,5] Benzoylecgonine and ecgonine methyl ester have been considered pharmacologically inactive when administered systemically.[6] However, recent *in vitro* and *in vivo* studies have shown that these metabolites are potent vasoconstrictors.[7–11]‖ In contrast to these reports, our recent observations indicate that intravenously administered benzoylecgonine does not alter the behavior or the hemodynamic state of the rat,[12] whereas cocaine, norcocaine, or cocaethylene in doses equivalent to benzoylecgonine caused neurobehavioral changes.[13],#,**

Based on our preliminary observations we hypothesized that, at doses equal to cocaine, benzoylecgonine and ecgonine methyl ester do not cause central nervous system or systemic hemodynamic sequelae. To test this hypothesis, we performed a comparative toxicity study of cocaine and its metabolites in the rats fitted with catheters for long-term monitoring.

Materials and Methods

Animals

The study protocol was approved by the Columbia University Animal Care and Use Committee. Forty-two nonpregnant female Sprague-Dawley rats were purchased from a commercial breeder (CAMM, Ridgefield, NJ) 1 week before the surgery. They were housed in a temperature-controlled room and fed laboratory rodent

* Professor of Anesthesiology.

† Assistant Professor of Anesthesiology.

‡ Postdoctoral Research Fellow in Anesthesiology.

§ Professor of Psychiatry.

Received from the Departments of Anesthesiology and Psychiatry, College of Physicians and Surgeons, Columbia University, New York, New York. Submitted for publication August 12, 1998. Accepted for publication January 29, 1999. Supported in part by the National Institute on Drug Abuse, grant R01 DA06648, and by the National Institute of Mental Health, grant MHCRC 30906, Bethesda, Maryland.

Address reprint requests to Dr. Morishima: Department of Anesthesiology, College of Physicians and Surgeons, Columbia University, 622 West 168th Street, PH Box 9, New York, New York 10032. Address electronic mail to: hom2@columbia.edu

‖ Schreiber MD, Anaya C, Modipalli L, Torgerson LJ, Marks J: Cocaine and cocaine metabolite responses in newborn guinea pig cerebral arteries (abstract). Pediatr Res 1996; 39:457A.

Whittington RA, Iso A, Khan K, Cooper TB, Morishima HO: Polydrug toxicity: Cocaine and alcohol toxicity in rats (abstract). Anesth Analg 1995; 80:S553

** Whittington RA, Iso A, Khan K, Cooper TB, Morishima HO: Norcocaine is more toxic than cocaine in rats (abstract). ANESTHESIOLOGY 1996; 85:A767.

chow *ad libitum* with water. The animals were divided randomly into five study drug groups: cocaine (n = 10), norcocaine (n = 8), cocaethylene (n =8), ecgonine methyl ester (n = 8), and benzoylecgonine (n = 8).

Surgical Procedures

Surgical procedures have been described in detail elsewhere.[13,14] Briefly, 1–3 days before the toxicity study, the animals were anesthetized using an intraperitoneal injection of a xylazine–ketamine combination (6 mg/kg xylazine and 40 mg/kg ketamine). During sterile conditions, polyethylene catheters (PE 50; Becton Dickinson, Sparks, MD) were placed into the right carotid artery and jugular vein of each rat. The catheters were tunneled and secured outside the animals through a small skin incision made between the scapulae, and then they were coiled and covered with a plastic cap that was secured to the skin. After operation, the animals were allowed to recover from surgery for 1–3 days.

Experimental Procedures

On the day of the study, the awake rat was weighed and placed in a semidark, closed cardboard box with small windows on the sides instead of in the rat restraint holder. The catheters were threaded through a hole at the top of the box to allow the rat to move freely. The details regarding this awake, nonrestrained rat model also have been described before.[13,14]

Before the study was begun, each animal was allowed to acclimate to the new environment for at least 1 h. During that time, the arterial catheter was connected to a pressure transducer, and arterial blood pressure and heart rate were monitored subsequently during the study using a polygraph recorder (Gould Electronics, Rutherford, NJ) connected to a computer (Apple Computers, Cupertino, CA). The heart rate was monitored using a cardiotachometer triggered by the arterial pulse pressure. All compounds were supplied by the National Institute on Drug Abuse. In the toxicity study, after a 60- to 90-min stabilization period (baseline), cocaine or one of the metabolite solutions was infused continuously into the jugular vein at a rate of 2 mg $\cdot$ kg^{-1} $\cdot$ min^{-1} and the animal was observed for the onset of changes in behavioral and stereotypic locomotor activity and for more advanced central nervous system toxic manifestations, such as the onset of tonic–clonic convulsions. The details of behavioral changes have been described before.[13,14]

The infusion was continued until circulatory collapse occurred, which was defined as the disappearance of the arterial blood pressure and heart rate from the recorder. Initially, this infusion rate was chosen based on our previous cocaine studies.[13,14]‖# However, we observed in the preliminary studies that, at this infusion rate, benzoylecgonine and ecgonine methyl ester produced only a mild increase in locomotor activity but did not produce convulsions. Therefore, to reliably produce cardiovascular and neurologic toxic manifestations, the infusion rate for ecgonine methyl ester was increased incrementally to a final rate of 32 mg $\cdot$ kg^{-1} $\cdot$ min^{-1}. Furthermore, the infusion rate for benzoylecgonine was increased subsequently to a final study rate of 192 mg $\cdot$ kg^{-1} $\cdot$ min^{-1}, after this compound failed to produce advanced cardiovascular and neurologic toxic manifestations at a rate of 32 mg $\cdot$ kg^{-1} $\cdot$ min^{-1}.

Animal behavior was recorded every minute, and the onset of restlessness or agitation was defined in the following manner: after the baseline behavior was observed, the time was noted when the animal began to exhibit agitated movements, such as repetitive grooming behavior and circling.[13,14] If this agitated behavior persisted for 3 min, the first minute was defined as the time of onset of agitation. Similarly, the onset of depression was the beginning of an interval, lasting at least 3 min, of behavioral activity, whereby the animal showed no movement of the extremities and no response to the touching of earlobes or the nose. These behavioral changes were noted by the same highly experienced observer who has been performing behavioral studies for the past 12 yr. The observer was blinded to the compound selected.

Because of blood volume limitations and to avoid interference with the animal's behavior during the early stages of the response to the compound, 0.3-ml arterial blood samples were obtained only at the onset of major signs of intoxication, such as convulsions, hypotension, and circulatory collapse. Blood samples were collected in chilled, heparin-prepared syringes and transferred immediately into ice-cold Vacutainer tubes (Becton Dickinson) containing 17.5 mg sodium fluoride and 14 mg potassium oxalate. The samples were centrifuged at −0°C and the plasma layer was separated and stored at −70°C for subsequent drug assays. At the end of the experiment, the brain, heart, and liver were removed and placed immediately on dry ice. In addition, a urine sample was aspirated from the bladder and transferred into the Vacutainer tube containing sodium fluoride and potassium oxalate. Thereafter, these samples were stored at −70°C until subsequent drug assays were performed.

Special Analytic Procedures

All compounds were analyzed by capillary gas chromatography–mass spectrometry using deuterated internal standards, positive chemical ionization, and simultaneous ion monitoring.

Deuterated internal standards for cocaine, cocaethylene, norcocaine, benzoylecgonine, and ecgonine methyl ester were added to the plasma or tissue homogenate before processing. Deuterated cocaine and benzoylecgonine were purchased from Sigma Chemical Company (St. Louis, MO). Ethylbenzoylecgonine was purchased from Research Biochemicals (Natick, MA). Deuterated norcocaine and ecgonine methyl ester were donated by the National Institute on Drug Abuse, and deuterated ethylbenzoylecgonine was synthesized in our laboratory by forming the ethyl ester of trideuterobenzoylecgonine. The electron impact mass spectra of each of these compounds showed fragmentation patterns with appropriate mass unit shifts for the deuterated internal standard.

Depending on the nature of the study, the standard curves encompassed 0–500 ng/ml or g up to 10,000 ng/ml or g in the short studies. In the latter case, larger amounts of the internal standard were added. Standard curves for each of these compounds were linear throughout the entire range, with low intercepts and correlation coefficients of 0.999 or more. The intra- and intercoefficients of variation for these compounds were less than 6% across the entire range of the samples tested, and we found the procedure to be highly sensitive and reliable (lowest detectable quantity, 0.5 ng/ml). Quality control samples at low, medium, and high concentrations of cocaine and all metabolites were analyzed with each analytic run.

Statistical Analysis

Analysis of variance tests were performed using the null hypothesis and indicated that there were no significant differences in the mean values among the five groups of animals. The Scheffé *post hoc* test was used when appropriate. All results are expressed as the mean ± SE. P values < 0.05 were considered significant.

Results

The weights of all animals were similar, ranging from 235 g to 315 g, with a mean (± SE) of 261 ± 6.5 g. The baseline values for heart rate and mean arterial pressure were within the normal range for the rat and were similar in all groups of animals (table 1). All animals

Table 1. Body Weight, Baseline Heart Rate, and Mean Arterial Pressure

Toxicity Study	Weight (g)	Heart Rate (beats/min)	Blood Pressure (mmHg)
Cocaine (N = 10)	264 ± 9.2	432 ± 17.1	119 ± 3.7
Norcocaine (N = 8)	237 ± 1.1	436 ± 16.9	118 ± 7.8
Cocaethylene (N = 8)	274 ± 2.9	400 ± 30.2	124 ± 6.0
Ecgonine methyl ester (N = 8)	268 ± 8.9	420 ± 33.2	111 ± 4.9
Benzoylecgonine (N = 8)	263 ± 3.8	426 ± 0.7	108 ± 1.8

Values are mean ± SE.

receiving cocaine, norcocaine, or cocaethylene became restless within 5 min after the infusion began and displayed sniffing and repetitive grooming behavior. This hyperexcitability was followed by more violent types of agitation: the rats climbed up the walls, frequently chewed the edges of the cardboard box, and circled wildly. This behavior was accompanied by a 15–25% increase in mean arterial blood pressure and a 4–15% decrease in heart rate. An increase in blood pressure became more apparent when the animals subsequently became depressed during the infusion of cocaine and cocaethylene. Dissimilar to the animals that received cocaine and cocaethylene, none of the animals receiving norcocaine exhibited sedation or depression before convulsions began. The first sign of norcocaine toxicity was restlessness and a significant increase in blood pressure from the baseline value of 118 ± 9 mmHg to 149 ± 10 mmHg ($P < 0.05$), which was followed rapidly by tonic-clonic convulsions. Furthermore, the norcocaine-induced convulsions were more violent than those produced by cocaine or cocaethylene: the animals jumped up high and frequently projected themselves out of the 20-cm-high observation box. We never observed this degree of convulsant activity with cocaine- or cocaethylene-induced convulsions. At the onset of convulsions produced by cocaine, cocaethylene, and norcocaine, the mean arterial blood pressure increased significantly—approximately 40% from baseline—and this was accompanied by a 15% decrease in heart rate. For example, in the cocaine group, blood pressure increased from a baseline of 119 ± 4 mmHg to 166 ± 4 mmHg ($P < 0.001$), and heart rate decreased from 432 ± 17 beats/min to 353 ± 11 beats/min ($P < 0.005$).

Neurobehavioral and locomotor changes in the animals receiving ecgonine methyl ester and benzoylecgonine were different from those in animals in the cocaine, norcocaine, and cocaethylene groups. The animals ex-

Table 2. Dose (mg/kg) Required to Produce the Onset of Toxic Manifestations

	Excitement	Depression	Convulsions	Hypotension	Circulatory Collapse
Cocaine (N = 10)	8.8 ± 1.4	23.9 ± 2.1	24.8 ± 1.9	31.7 ± 1.8	35.2 ± 1.6
Norcocaine (N = 8)	3.1 ± 0.8	*	5.2 ± 0.6	7.4 ± 0.9	8.6 ± 1.1
Cocaethylene (N = 8)	8.3 ± 1.2	20.8 ± 0.4	21.9 ± 2.7	27.7 ± 2.2	28.6 ± 3.3
Ecgonine methyl ester (N = 8)	298.2 ± 49.6	319.6 ± 83.0	849.9 ± 61.6	1125.7 ± 111.5	1182.3 ± 97.9
Benzoylecgonine (N = 8)	96.0 ± 13.9	289.2 ± 92.2†	2841‡	4297.2‡	5677.8‡

Values are mean ± SE.

* None of animals exhibited sedation/depression during norcocaine infusion.

† Somnolence.

‡ Only one with the infusion rate of 192 mg · kg^{-1} · min^{-1}.

hibited a subtle degree of restlessness manifested by vertical and horizontal head swinging. This period was followed by somnolence. Convulsions in the ecgonine methyl ester group were characterized by occasional single bursts of tonic convulsions, rather than by the intermittent, vigorous tonic–clonic seizures observed in the cocaine and cocaethylene groups. The tonic–clonic convulsions that are typical of local anesthetic-induced excitotoxicity never developed in the animals in the benzoylecgonine group; however, with higher drug infusion rates (32–64 mg · kg^{-1} · min^{-1}), they exhibited myoclonic twitching. Blood pressure and heart rate in these animals remained essentially unaltered during the infusion; hypotension and bradycardia occurred in only one animal in this group.

Table 2 summarizes the doses necessary to produce all toxic manifestations. Each toxic manifestation began at similar doses in the cocaine and cocaethylene groups. For example, convulsions occurred at a dose of 24.8 ± 1.9 mg/kg in the animals receiving cocaine, whereas they occurred at a dose of 21.9 ± 2.7 mg/kg in the animals that received cocaethylene. The animals that received norcocaine became restless within 2 or 3 min of the infusion at a dose of 3.1 ± 0.8 mg/kg, which was approximately 33% of the cocaine or cocaethylene dose necessary to produce similar toxic manifestations. In contrast, extremely large doses of ecgonine methyl ester and benzoylecgonine were necessary for the animals to develop apprehensive behavior, hyperexcitability, and depression; a benzoylecgonine infusion rate 15–30 times greater than that of cocaine produced only subtle jerking movements that occurred during sleep. Further increases in the infusion rate to 160 times greater than the lethal dose of cocaine resulted in circulatory collapse in only one animal.

Table 3 summarizes the plasma concentrations of each compound and its metabolites at the onset of each toxic manifestation. The major metabolite of cocaine, cocaethylene, and norcocaine was benzoylecgonine. For example, at the onset of cocaine-induced convulsions, the plasma cocaine concentration was approximately 70% of the total detected compound, and the metabolites were distributed as follows: benzoylecgonine: 26%; ecgonine methyl ester: 3 to 4%; and norcocaine: 2 to 3%. Although the dose of norcocaine necessary to produce toxic manifestations was significantly less than cocaine or cocaethylene ($P < 0.01$), plasma concentrations of these three compounds at the onset of each toxic manifestation were similar. Norcocaine administration resulted in the formation of a small amount of norbenzoylecgonine, 3 or 4% of the parent compound: 0.62 ± 0.31 μg/ml at the onset of convulsions, and 2.37 ± 0.73 μg/ml at circulatory collapse.

The tissue concentration patterns of the infused compounds in the brain and heart were similar in the animals receiving cocaine, norcocaine, and cocaethylene (table 4), and the concentrations of the infused compounds were significantly greater compared with those for the liver ($P < 0.001$). In contrast, the animals in the benzoylecgonine and ecgonine methyl ester groups had greater concentrations of the infused compounds in the liver than in the brain ($P < 0.001$). The norbenzoylecgonine concentration in the brain, heart, and liver after norcocaine administration was 4.29 ± 1.05, 6.31 ± 1.36, and 6.31 ± 1.37 μg/g, respectively. In the animals receiving cocaine, the parent compound was excreted in the urine, amounting to 107.9 ± 18.6 μg/ml, whereas benzoylecgonine was the major metabolite, amounting to 44.1 ± 5.9 μg/ml.

Discussion

This *in vivo* comparative toxicity study was designed to evaluate the tissue distribution of systemically administered cocaine and its metabolites in conscious, pharmacologi-

Table 3. Plasma Concentrations (μg/ml) of Parent Compound and Metabolites at the Onset of Toxic Manifestations

Infused Compound	Cocaine	Benzoylecgonine	Ecgonine Methyl Ester	Norcocaine	Cocaethylene
Convulsions					
Cocaine (N = 10)	9.30 ± 1.08*	3.55 ± 0.43	0.43 ± 0.08	0.27 ± 0.03	ND
Norcocaine (N = 8)	ND	ND	ND	7.57 ± 0.36*	ND
Cocaethylene (N = 8)	0.02 ± 0.001	1.47 ± 0.28	ND	ND	10.78 ± 0.54*
Ecgonine methyl ester (N = 8)	ND	ND	362.22 ± 33.25*	ND	ND
Benzoylecgonine (N = 8)	54.00 ± 6.00†	702.76 ± 243.24*†	ND	ND	ND
Hypotension					
Cocaine (N = 10)	14.69 ± 1.90*	4.53 ± 0.29	0.62 ± 0.09	0.35 ± 0.03	ND
Norcocaine (N = 8)	ND	ND	ND	10.98 ± 1.85*	ND
Cocaethylene (N = 8)	0.03 ± 0.001	1.41 ± 0.15	ND	ND	13.32 ± 1.18*
Ecgonine methyl ester (N = 8)	ND	ND	446.65 ± 74.63*	ND	ND
Benzoylecgonine (N = 8)	NA	NA	NA	NA	NA
Circulatory collapse					
Cocaine (N = 10)	54.76 ± 8.37*	10.95 ± 1.14	1.21 ± 0.14	0.74 ± 0.10	ND
Norcocaine (N = 8)	ND	ND	ND	41.00 ± 2.44*	ND
Cocaethylene (N = 8)	0.06 ± 0.23	2.61 ± 0.25	ND	ND	38.62 ± 9.29*
Ecgonine methyl ester (N = 8)	ND	ND	723.24 ± 63.99*	ND	ND
Benzoylecgonine (N = 8)	NA	NA	NA	NA	NA

Values are mean ± SE.

ND = not detected; NA = not applicable.

* Infused compound.

† Sample taken during sleep.

cally naive rats. Our results indicate that cocaine and cocaethylene, when administered intravenously, produce similar systemic toxic effects at comparable doses and plasma and tissue concentrations. These findings correspond with those of studies reported by other investigators in conscious and anesthetized rats and monkeys in which cocaethylene produced effects similar to those of cocaine at approximately equivalent doses.[15–18] *In vitro*, the study of

Table 4. Tissue Concentrations (μg/g) of Parent Compound and Metabolites at the Onset of Circulatory Collapse

Infused Compound	Cocaine	Benzoylecgonine	Ecgonine Methyl Ester	Norcocaine	Cocaethylene
Brain					
Cocaine (N = 10)	90.01 ± 7.83*	3.05 ± 0.34	1.28 ± 0.08	2.17 ± 0.20	ND
Norcocaine (N = 8)	ND	ND	ND	71.11 ± 5.22*	ND
Cocaethylene (N = 8)	0.49 ± 0.002	0.83 ± 0.04	ND	ND	75.20 ± 5.57*
Ecgonine methyl ester (N = 8)	ND	ND	478.43 ± 57.89*	ND	ND
Benzoylecgonine (N = 8)	0.27 ± 0.06†	111.32 ± 68.45*†	ND	ND	ND
Heart					
Cocaine (N = 10)	100.44 ± 12.04*	6.59 ± 0.42	2.84 ± 0.25	2.39 ± 0.26	ND
Norcocaine (N = 8)	ND	ND	ND	108.45 ± 15.93*	ND
Cocaethylene (N = 8)	0.14 ± 0.02	2.59 ± 0.33	ND	ND	77.31 ± 8.45*
Ecgonine methyl ester (N = 8)	ND	ND	1748.22 ± 92.65*	ND	ND
Benzoylecgonine (N = 8)	0.27 ± 0.15†	1362.57 ± 759.11*†	ND	ND	ND
Liver					
Cocaine (N = 10)	25.34 ± 3.44*	14.66 ± 1.49	11.70 ± 1.23	7.39 ± 0.77	ND
Norcocaine (N = 8)	ND	ND	ND	25.53 ± 7.01*	ND
Cocaethylene (N = 8)	0.04 ± 0.001	8.42 ± 1.28	ND	ND	13.68 ± 4.29*
Ecgonine methyl ester (N = 8)	ND	ND	2150.11 ± 172.32*	ND	ND
Benzoylecgonine (N = 8)	0.04 ± 0.001†	1298.44 ± 687.88*†	0.15 ± 0.04	ND	0.35 ± 0.12

Values are mean ± SE.

ND = not detected.

* Infused compound.

† Sample taken during sleep.

the sodium channel blocking properties of the major metabolites of cocaine in single cardiac myocytes also indicate that benzoylecgonine and ecgonine methyl ester are ineffective blockers at clinically relevant concentrations.[19] Furthermore, smaller doses of norcocaine are necessary to produce lethal effects compared with cocaine or cocaethylene, although benzoylecgonine and ecgonine methyl ester are not as toxic as has been speculated. In addition, the latter two major polar metabolites penetrate poorly into brain tissue, so the acute intravenous administration of benzoylecgonine may not produce brain concentrations high enough to cause central nervous system toxicity.

Norcocaine, a pharmacologically active cocaine metabolite formed *via* N-demethylation by hepatic microsomal enzymes, has been shown to possess activity similar to cocaine in its ability to inhibit norepinephrine reuptake in brain synaptosomes.[1] As expected, the proportional concentration of norcocaine in the liver was greater (13%) than that found in other tissues, in which norcocaine accounted for only 1 or 2% of the total amount of the compounds measured. In addition, norcocaine and its metabolite, norcocaine N-oxide, may play a role in cocaine-induced hepatotoxicity.[20] Despite the observation that norcocaine produced circulatory collapse at a smaller dose, the plasma and tissue concentration at each toxic end point did not differ from cocaine or cocaethylene, suggesting a greater bioavailability of this compound. An earlier report[5] documented that norcocaine is more potent than cocaine, but the brain–plasma concentration ratios of both compounds at the same dose were similar.

Furthermore, when equimolar concentrations of cocaine and norcocaine were infused intravenously into the conscious rat, norcocaine produced convulsions and respiratory arrest earlier than did cocaine, yet the plasma concentrations of both compounds were similar.[21] Unfortunately, these investigators did not collect samples during convulsions to correlate plasma drug concentrations with central nervous system excitotoxicity. In addition, plasma norcocaine and ecgonine methyl ester could not be detected in almost all of the samples obtained during the cocaine infusion. In contrast, in the current study, all samples obtained at the onset of cocaine-induced convulsions, hypotension, or circulatory collapse had substantial plasma concentrations of the metabolites of cocaine, indicating that this difference is clearly related to the sensitivity of the analytic method. High-performance liquid chromotagraphy was the analytic method used in the other study,[21] and the detection limit for cocaine and norcocaine was 80 ng/ml; for benzoylecgonine it was 35 ng/ml; and for ecgonine methyl ester it was 100 ng/ml. However, we used gas chromatography–mass spectrometry in our study with sensitivities of 1 ng/ml for cocaine, norcocaine, and cocaethylene, and 0.5 ng/ml for benzoylecgonine and ecgonine methyl ester.

We might argue that the comparisons of *in vivo* toxicity in cocaine and its metabolites by administering an equivalent dose rather than an equimolar dose is inappropriate, because the molecular weight of cocaine is 339.8 and for ecgonine methyl ester it is 235.1 daltons. However, despite this difference, the dose of ecgonine methyl ester to produce each toxic manifestation was more than 100 times greater than that of cocaine, norcocaine (molecular weight, 289.3), benzoylecgonine (molecular weight, 289.3) and cocaethylene (molecular weight, 491.5). Consequently, this argument is not likely to be a potentially important issue in the current study.

Whether benzoylecgonine and ecgonine methyl ester are also pharmacologically active has not been shown. An early report suggested that these metabolites were significantly less toxic than cocaine or norcocaine.[6] In contrast, recent *in vitro* studies showed that benzoylecgonine and cocaine constrict isolated cerebral arteries in animals, whereas ecgonine methyl ester caused mild vasodilatation.[7,10]‖ When cocaine and its metabolites were infused directly over the brain surface, norcocaine decreased the arteriolar diameter significantly more than benzoylecgonine or ecgonine methyl ester did.[8] Despite these potentially important *in vitro* and *in vivo* findings, no clear data exist with regard to the tissue concentration of benzoylecgonine or ecgonine methyl ester necessary to cause such complications or with regard to the cocaine dose needed to form a toxic concentration of these metabolites.

In the current experiments, we failed to produce the typical pattern of cocaine-induced alterations in systemic arterial blood pressure and heart rate after the administration of these metabolites at plasma concentrations more than 100 times greater than that formed after the administration of a toxic dose of cocaine. In addition, circulatory collapse did not occur in the animals receiving extremely high benzoylecgonine doses. Another potentially important finding was that the distribution of these two metabolites in the brain tissue was significantly less than that found in the heart or liver. These results suggest that a significant "blood–brain barrier" exists with respect to these polar metabolites. A similar trend was observed in the "blood–placental barrier" when benzoylecgonine was administered to the preg-

nant rat[12]; a markedly high concentration of this metabolite remained in the maternal side of the placenta compared with the fetal side of the placenta. Given the poor penetration of these metabolites to brain tissue, it may not be appropriate to extrapolate the findings of *in vitro* studies *in vivo*. When the brain is exposed directly to benzoylecgonine *via* an intracisternal injection, only 1 mg/kg benzoylecgonine caused central nervous system hyperexcitability, whereas intravenous administration of 250 mg/kg produced no observable pharmacologic effects in the rat.[5] A more recent report indicates that benzoylecgonine, when injected intrathecally in small doses, produces a greater seizure frequency than cocaine does in awake rats,[22] indicating the importance of the route of administration when the toxicity of cocaine is compared with that of its metabolites.

In conclusion, this comparative toxicity study in the awake rat indicates that the major metabolites of cocaine, benzoylecgonine and ecgonine methyl ester, are unlikely to be as harmful as previously believed when administered systemically. The relative uptake of these polar metabolites in the brain is less than that of the parent compound. Although a smaller dose of norcocaine than of cocaine is needed to produce lethal effects, the amount of this metabolite, formed after cocaine is administered acutely to the rat, did not reach concentrations great enough to produce toxic effects. Further study is necessary to explore the mechanisms of the neurotoxic effects of benzoylecgonine and ecgonine methyl ester in subjects exposed to cocaine for long periods, because the elimination half-lives of benzoylecgonine and ecgonine methyl ester are significantly longer than that of cocaine.[23,24]

The authors thank K. Khan, B.S., B. Katof, M. Meister, and D. Allen for their technical assistance.

References

1. Hawks RL, Kopin IJ, Colburn RW, Thoa N: Norcocaine: A pharmacologically active metabolite of cocaine found in the brain. Life Sci 1974; 15:2189–95

2. Inaba T, Stewart DJ, Kalow W: Metabolism of cocaine in man. Clin Pharmacol Ther 1978; 23:547–52

3. Stewart DJ, Inaba T, Tang K, Kalow W: Hydrolysis of cocaine in human plasma cholinesterase. Life Sci 1977; 20:1557–63

4. Kloss MW, Rosen GM, Rauckman E: N-demethylation of cocaine to norcocaine. Evidence for participation by cytochrome P-450 and FAD-containing monooxygenase. Mol Pharmacol 1983; 23:482–5

5. Misra AL, Pontani RB, Vadlamani NL: Metabolism of norcocaine, N-hydroxynorcocaine, and cocaine-N-oxide in the rat. Xenobiotica 1979; 9:189–99

6. Misra AL, Nayak PK, Bloch R, Mule SJ: Estimation and disposition of [3H]benzoylecgonine and pharmacological activity of some cocaine metabolites. J Pharm Pharmac 1975; 27:784–6

7. Madden JA, Powers RH: Effect of cocaine and cocaine metabolites on cerebral arteries in vitro. Life Sci 1990; 47:1109–14

8. Kurth CD, Monitto C, Luiza M, Albuquerque ML, Feula P, Anday E, Shaw L: Cocaine and its metabolites constrict cerebral arterioles in newborn pigs. J Pharmacol Exper Ther 1993; 265:587–91

9. Covert RF, Schreiber MD, Tebbett IR, Torgerson LJ: Hemodynamic and cerebral blood flow effects of cocaine, cocaethylene and benzoylecgonine in conscious and anesthetized fetal lambs. J Pharmacol Exper Ther 1994; 270:118–25

10. Schreiber MD, Madden JA, Covert RF, Torgerson LJ: Effects of cocaine, benzoylecgonine, and cocaine metabolites on cannulated pressurized fetal sheep cerebral arteries. J Appl Physiol 1994; 77:834–9

11. Brogan WC III, Lange RA, Glamann B, Hillis LD: Recurrent coronary vasoconstriction caused by intranasal cocaine: Possible role for metabolites. Ann Intern Med 1992; 116:556–61

12. Morishima HO, Whittington RA, Khan K, Okutomi T, Cooper TB: The maternal to fetal transfer of benzoylecgonine. ANESTHESIOLOGY 1997; 87:A879

13. Morishima HO, Abe Y, Matsuo M, Akiba K, Masaoka T, Cooper TB: Gender-related difference in cocaine toxicity in the rat. J Lab Clin Med 1993; 122:157–63

14. Morishima HO, Masaoka T, Hara T, Tsuji A, Cooper TB: Pregnancy decreases the threshold for cocaine-induced convulsions in the rat. J Lab Clin Med 1993; 122:748–56

15. Tella SR, Korupolu GR, Schindler CW, Goldberg SR: Pathophysiological and pharmacological mechanisms of acute cocaine toxicity in conscious rats. J Pharmacol Exp Ther 1992; 262:936–46

16. Hearn WL, Rose S, Wagner JA, Mash DC: Cocaethylene is more potent than cocaine in mediating lethality. Pharmacol Biochem Behav 1991; 39:531–3

17. Erzouki HK, Baum I, Goldberg SR, Schindler CW: Comparison of effects of cocaine and its metabolites on cardiovascular function in anesthetized rats. J Cardiovasc Pharmacol 1993; 22:557–63

18. Schindler CW, Zheng J, Tella SR, Goldberg SR: Comparison of the effects of cocaine and cocaethylene on cardiovascular function in squirrel monkeys, Problems of Drug Dependence. Edited by Harris L. NIDA Monograph Series 132. Washington, DC, US Government Printing Office, 1993, p 367

19. Crumb WJ Jr, Clarkson CW: Characterization of the sodium channel blocking properties of the major metabolites of cocaine in single cardiac myocytes. J Pharmacol Exper Ther 1992; 261:910–7

20. Rauckman EJ, Rosen GM, Cavagnaro J: Norcocaine nitroxide. A potential hepatotoxic metabolite of cocaine. Mol Pharmacol 1982; 21:458–63

21. Mets B, Virag L: Lethal toxicity from equimolar infusions of cocaine and cocaine metabolites in conscious and anesthetized rats. Anesth Analg 1995; 81:1033–8

22. Konkol RJ, Erickson BA, Doerr JK, Hoffman RG, Madden JA: Seizures induced by the cocaine metabolite benzoylecgonine in rats. Epilepsia 1992; 33:420–7

23. Ambre J, Ruo TI, Nelson J, Belknap S: Urinary excretion of cocaine, benzoylecgonine, and ecgonine methyl ester in humans. J Anal Toxicol 1988; 12:301–6

24. Ambre JJ, Connelly TJ, Ruo T-I: A kinetic model of benzoylecgonine disposition after cocaine administration in humans. J Anal Toxicol 1991; 15:17–20