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The Effect of Postmortem Interval on the Concentrations of Cocaine and Cocaethylene in Blood and Tissues: An Experiment Using Rats

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ABSTRACT: Cocaine and cocaethylene concentrations in blood and tissues at early stages postmortem (0–6 h) were investigated using alcohol-treated rats. Gas chromatography/mass spectrometry following a liquid/liquid extraction procedure was employed to detect these drugs. Calibration curves showed good linearity in the range of 0 to 2,500 ng/mL with correlation coefficients of 0.9999 and 0.9998 for cocaine and cocaethylene, respectively. In a group treated with cocaine and ethanol orally, the liver lost over 25% of the cocaine present at death after 1 h. Conversely, the hepatic cocaethylene concentrations at this time reached more than twice those at death. Thereafter, the hepatic concentrations of cocaine and cocaethylene were maintained at a constant level until 6 h postmortem. Similar results were obtained with rats given cocaine intramuscularly. No changes in the cocaine and cocaethylene concentrations in any other tissues during the 6-h of postmortem period were observed. The forensic pathologist and toxicologist should be aware of these phenomena when selecting postmortem specimens for the analysis of cocaine and cocaethylene and take them into account when interpreting the results.

KEYWORDS: forensic science, forensic toxicology, toxicology, cocaine, cocaethylene, postmortem stability of cocaine, drug-alcohol interactions, gas chromatography/mass spectrometry, animal model, rat

It is very important for the forensic toxicologist to select appropriate specimens for the determination and interpretation of drug levels postmortem, because the concentrations of basic drugs in a specimen tend to increase with time after death (1–4). For example, tricyclic antidepressants (1–3), narcotic analgesics (1,2,4), local anesthetics (2) and antihistamines (2) that accumulate in myocardial tissue lead to elevated levels in heart blood postmortem.

Postmortem degradation of a drug also interferes with the accurate interpretation of intoxication in the deceased. Cocaine is converted rapidly into benzoylecgonine or ecgonine methyl ester in liver and blood of a living person (5). Metabolism of liver cocaine is mediated at least by two nonspecific hepatic esterases; one produces benzoylecgonine and the other produces ecgonine methyl ester (6). Blood cocaine is converted spontaneously into benzoylecgonine and is metabolized to ecgonine methyl ester by plasma pseudocholinesterase (7). Thus, cocaine is broken down gradually also in postmortem specimens by residual esterase activities and putrefaction (7–10). It was demonstrated experimentally that cocaethylene, an active metabolite of cocaine, was formed in the presence of cocaine and ethanol by the hepatic esterase which produce benzoylecgonine in the absence of ethanol (6). Hearn et al. (11) reported that, in their autopsy cases positive for both cocaine and cocaethylene, many showed higher tissue levels of cocaethylene than cocaine, especially in the liver. However, whether cocaethylene is produced after death by residual hepatic esterase activity remains to be evaluated. The purpose of this study was to investigate cocaine and cocaethylene concentrations in blood and tissues of ethanol-treated rats during the early stages postmortem using gas chromatography/mass spectrometry (GC/MS).

Materials and Methods

Apparatus

A Shimadzu GC/MS 9020-DF (Kyoto, Japan) equipped with a TC-17 capillary column [50% phenylmethylsilicone, 15 m × 0.53 mm I.D., 1.0 μm film thickness (GL Science, Tokyo, Japan)] was used for the quantitation of cocaine and cocaethylene. The injection port temperature was 280°C and the column temperature was programmed as follows: an initial temperature of 220°C was maintained for 4 min, increased to 250°C at a rate of 10°C/min and this final temperature was maintained for 1 min. The temperatures of the separator and ion source were 280 and 270°C, respectively, the EI energy and accelerating voltage were 70 eV and 3.0 kV, respectively and the carrier gas was helium at a flow rate of 5 mL/min.

Reagents

Cocaine hydrochloride was purchased from Takeda Chemical Industries (Osaka, Japan). Benzoylecgonine was prepared from cocaine hydrochloride by published procedures (12,13). Cocaethylene and propylbenzoylecgonine were synthesized from benzoylecgonine in our laboratory using the method described by Bailey (14). All the other chemicals used were of analytical grade.

Blood samples containing 0, 125, 250, 625, 1,250 and 2,500 ng/mL cocaine and cocaethylene were used as calibration standards.

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Animal Experiment

Male Wistar rats weighing 250–300 g were used and treated as follows: a) 10 mL/kg aqueous solution containing 0.224% w/v cocaine hydrochloride (0.2% w/v as cocaine base) and 20% w/v ethanol was administered into the stomachs or b) 2 mL/kg physiological saline containing 1.12% w/v cocaine hydrochloride (1% w/v as cocaine base) was injected into the left femoral muscle immediately after oral administration of 10 mL/kg 20% w/v ethanol solution. The rats were sacrificed by dislocating cervical vertebrae 0.25 and 1 h after being treated with cocaine orally and intramuscularly, respectively, and left at 20–25°C. The large blood vessels around the heart were occuluded with Kocher clamps to prevent influx of blood into the heart ventricles. The portal veins of the group given oral cocaine were also occuluded with these clamps to protect the liver from contamination with mesenteric venous blood containing a high concentration of cocaine. The liver, brain and femoral muscle were collected 0, 1, 3 and 6 h after death and heart blood was collected 0 and 6 h postmortem.

Processing of Blood, Liver, Brain and Femoral Muscle for GC/MS Quantitation of Cocaethylene and Cocaine

An aliquot (0.5 mL) of each blood sample was placed in a glass test tube containing 0.5 mL 1.5% w/v sodium fluoride solution and kept on ice. A portion of each liver, brain and femoral muscle was homogenized in 3 portions of 1% w/v sodium fluoride solution in a glass test tube and kept on ice. One milliliter of blood mixture or 1 g homogenate was mixed with 50 μL 11 μg/mL propylbenzoylecgonine in methanol (internal standard) and 2 mL 1 M carbonate buffer (pH 9.7). Each mixture was extracted with 6 mL n-chlorobutane/isoamyl alcohol (98/2 v/v) for 15 min using a mechanical shaker and centrifuged at 2,500 rpm for 5 min. The organic phase was back-extracted with 1 mL 0.1 N HCl for 30 s using a vortex mixer and centrifuged at 2,500 rpm for 5 min. The resulting aqueous phase was washed with 4 mL 2-methylbutane/toluene/isoamyl alcohol (94/5/1 v/v/v), mixed with 2 mL carbonate buffer, then reextracted with 4 mL 2-methylbutane/toluene/isoamyl alcohol (94/5/1 v/v/v) for 30 s using the vortex mixer, centrifuged at 2,500 rpm for 5 min and this organic phase was evaporated to dryness at 50°C using a gentle stream of nitrogen gas. The residues were reconstituted with 50 μL methanol and a 1-μL aliquot of each mixture was injected into the GC/MS. Mass fragmentography of cocaine, cocaethylene and propylbenzoylecgonine was performed at m/z 182, 196 and 210, respectively.

Results

Cocaine and Cocaethylene Assays

Mass fragmentography yielded retention times of 5.3, 5.8 and 6.6 min for cocaine, cocaethylene and propylbenzoylecgonine, respectively. No interfering peaks were observed in the biological specimens. The respective average recoveries (n = 3) of the isolation procedure and relative standard deviations (n = 3) of the mass fragmentography, which were investigated using standard calibration blood samples containing 2,500 ng/mL cocaine and cocaethylene, were 65 and 0.90% for cocaine and 75 and 2.02% for cocaethylene. Calibration curves, which were prepared by plotting the concentrations of cocaine and cocaethylene versus peak area ratios of cocaine/propylbenzoylecgonine and cocaethylene/propylbenzoylecgonine of standard spiked blood samples, showed good linearity over the range of 0 to 2,500 ng/mL with correlation coefficients of 0.9999 and 0.9998 for cocaine and cocaethylene, respectively. The lower detection limits of the GC/MS quantitation method for both cocaine and cocaethylene in the blood and tissues were about 10 ng/mL and 30 ng/g, respectively.

Animal Experiment

In rats given cocaine orally, the average hepatic concentrations of cocaine and cocaethylene at death were high, 2,400 and 662 ng/g, respectively, with a large intersubject variation. Each liver had lost over 25% of its initial cocaine content 1 h after death, which resulted in elevated hepatic cocaethylene concentrations of over twice the initial values. Thereafter, the hepatic concentrations of cocaine and cocaethylene were maintained at a constant level until 6 h postmortem (Fig. 1 and Table 1).

A similar phenomenon was observed in rats given cocaine intramuscularly. Although the intramuscular group had a low mean hepatic cocaine concentration, 133 ± 25 ng/g, and no cocaethylene was detected in the liver at death, a detectable amount of cocaethylene, 42 ± 11 ng/g, was present in the liver 1 h after death. No further increases in the hepatic cocaethylene concentration were observed 3 and 6 h postmortem. The mean hepatic cocaine concentration ratio at 6 to 0 h postmortem was 0.67 (Fig. 2 and Table 2).

In other postmortem tissues, including blood, from both groups, no significant changes in the cocaine and cocaethylene levels over the 6-hour study period were observed (Figs. 1 and 2 and Tables 1 and 2).

Discussion

It is well known that cocaine is broken down significantly not only to ecgonine methyl ester by residual pseudocolinesterase activity in isolated blood specimens, but also to benzoylecgonine by spontaneous hydrolysis under alkaline conditions (7,10). Price (8) reported that the cocaine content of liver stored at 4°C for 2 months declined to one-tenth its initial level. Liu et al. (9) also reported that blood spiked with cocaine lost about 30% of the drug during storage at 16°C for 36 days. Hearn et al. (15) demonstrated that blood cocaine concentrations changed significantly during the interval between death and autopsy and that the direction of the change appeared to be dependent upon the site from which the blood is sampled.

Cocaethylene, an active metabolite of cocaine, is formed in the presence of cocaine and ethanol by the liver esterase which converts

TABLE 1—*Ratios of cocaine and cocaethylene concentrations in blood and tissues at each time after death to those at death in rats sacrificed 0.25 h after oral administration of 20 mg/kg cocaine and 2 g/kg ethanol.*

Samples	Drugs	Concentration ratios 0	1	3	6 h
Blood*	Cocaine	1.00	—	—	1.05±0.08
	Cocaethylene	1.00	—	—	1.29±0.27
Liver	Cocaine	1.00	0.63±0.09**	0.61±0.11**	0.54±0.04**
	Cocaethylene	1.00	2.75±0.51**	2.89±0.72**	2.62±0.53**
Brain	Cocaine	1.00	0.94±0.10	1.09±0.06	1.05±0.04
	Cocaethylene	1.00	1.03±0.12	1.05±0.11	1.07±0.18
Muscle	Cocaine	1.00	1.06±0.02	1.10±0.13	1.17±0.15
	Cocaethylene	1.00	1.05±0.02	1.10±0.11	1.18±0.14

Each value represents the mean of 5 animals ± S.D.
*:Blood samples were taken 0 and 6 h since death.
**:Statistically significant compared with the value at death (Student's t test, $P < 0.05$).

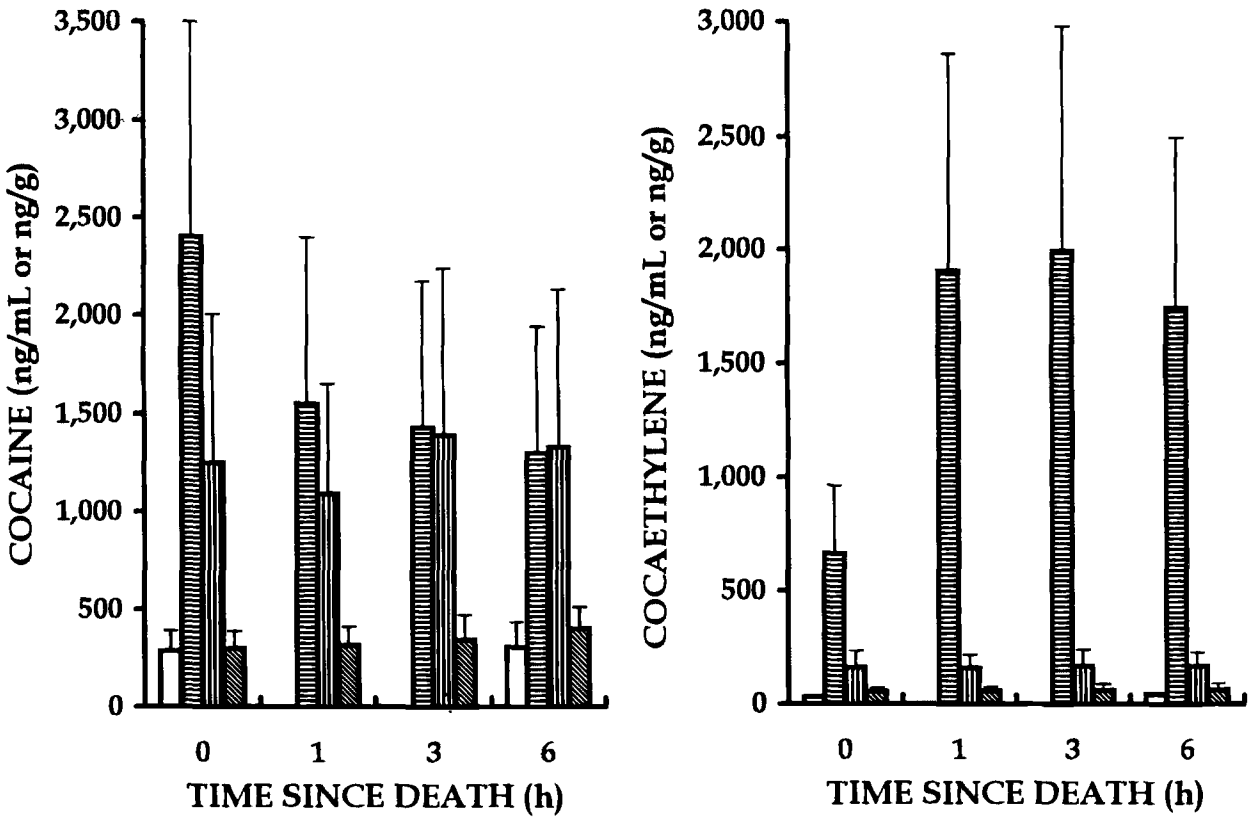

FIG. 1—*Cocaine and cocaethylene concentrations in postmortem blood (□), liver (▤), brain (▥) and muscle (▧) of rats given 20 mg/kg cocaine and 2 g/kg ethanol orally. The animals were sacrificed 1 h after the cocaine treatment. Blood samples were taken from the heart 0 and 6 h after death. Each bar represents the mean of 5 animals + S.D.*

cocaine into benzoylecgonine in the absence of ethanol (6). Cocaethylene is just as effective at blocking dopamine reuptake as cocaine in rats and produces the same behavioral alterations as cocaine in rhesus monkeys (16). Moreover, cocaethylene is as hepatotoxic as cocaine (17) and has a much lower LD_{50} than cocaine in mice (18). Thus, cocaethylene and cocaine should be analyzed simultaneously to ensure accurate interpretation of cocaine poisoning in decedents associated with antemortem usage of both cocaine and ethanol (19). However, whether significant changes in the concentrations of cocaine and cocaethylene in body fluids and tissues occur during the early stages postmortem have not fully been clarified.

In the present study on alcohol-treated rats, hepatic cocaethylene concentrations increased significantly 1 h postmortem both in the groups that received cocaine orally and intramuscularly, although no changes in its concentrations in any other tissues, including blood, were observed and cocaine concentrations declined only in the liver at this time. We confirmed that rat liver left at room temperature for 6 h after sacrifice was able to produce cocaethylene when its homogenates were incubated with cocaine and ethanol at 37°C (data not shown). Thus, the cessation of cocaethylene production in the liver 1 h after death might result from lowered activity of the hepatic esterase due to cooling of the rat carcasses.

Postmortem elevation of hepatic cocaethylene concentration in the oral group with a high hepatic cocaine concentration was much greater than that in the intramuscular group with a low hepatic cocaine concentration. This can be explained by the fact that rapid production of cocaethylene in the liver of living rats requires a relatively high concentration of liver cocaine in the presence of ethanol (20).

Intramuscular administration of cocaine into the alcohol-intoxicated rats resulted in no detection of cocaethylene in the liver at death, although in the other tissues cocaethylene was detected. Cocaethylene concentrations in the brain and muscle were much higher than that in the blood. These phenomena are a little puzzling because the liver is the main organ that can produce cocaethylene in the presence of cocaine and ethanol. Possible reasons for the phenomena are: 1) after its production, cocaethylene may escape rapidly into blood circulation from the liver without accumulating; 2) cocaethylene in the blood could easily be distributed to the brain and muscle; and 3) rat kidney as well as mouse kidney might have the ability to produce significant amounts of cocaethylene (21).

Cocaethylene produced postmortem in the liver may distribute readily into the blood due its very poor deposition in hepatic tissue (20). Although the data are not shown in this paper, elevated levels of cocaethylene in postmortem blood of rats treated orally with cocaine and ethanol were observed when more than 1 mL blood was taken from the heart without occuluding the large blood vessels. Thus, the sites from which blood samples are taken should also be taken into consideration and care taken to ensure appropriate samples are selected when analyzing cocaethylene, because extensive movement of body fluids through the vascular system may occur postmortem (22).

Conclusion

We have demonstrated that cocaine is transformed postmortem into cocaethylene in the liver of alcohol-intoxicated rats and that the formation of cocaethylene ceases approximately 1 h after death

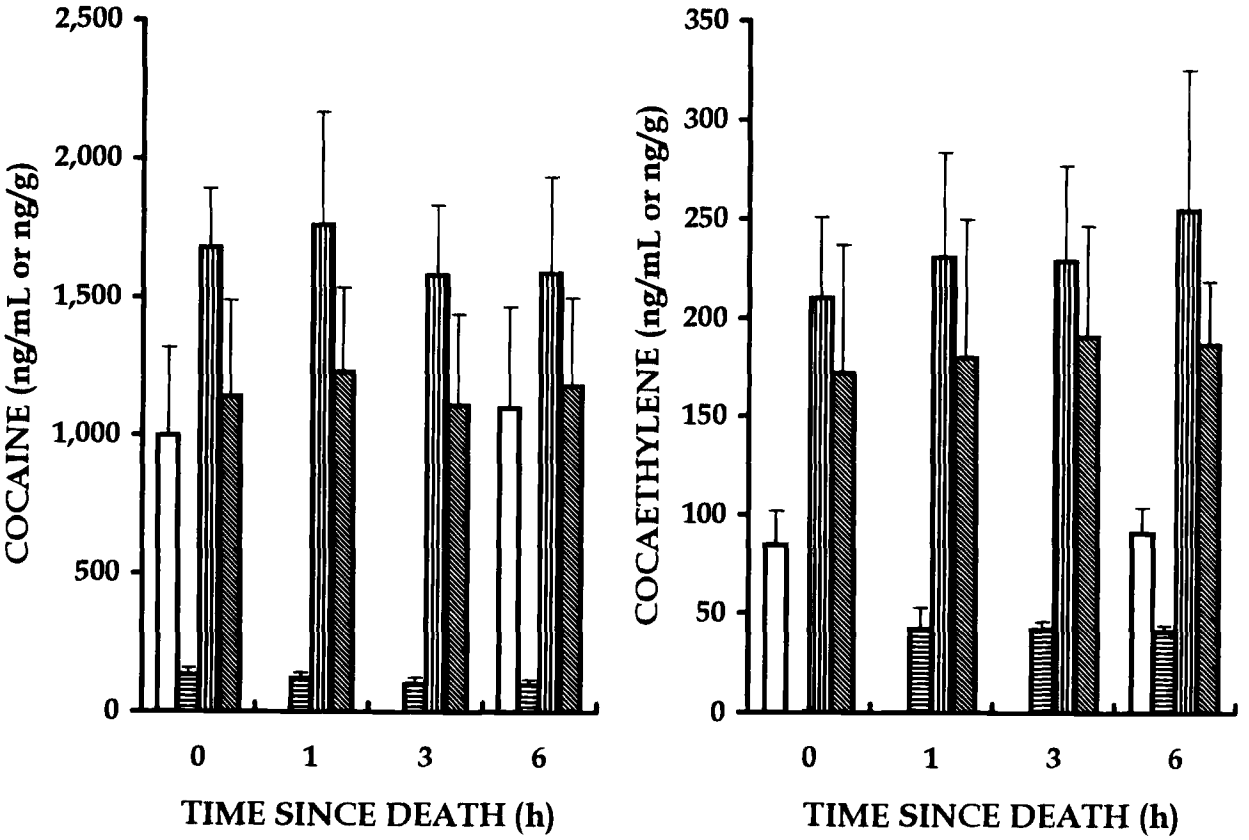

FIG. 2—*Cocaine and cocaethylene concentrations in postmortem blood (□), liver (▤), brain (▥) and muscle (▧) of rats given 20 mg/kg cocaine intramuscularly immediately after oral administration of 2 g/kg ethanol. The animals were sacrificed 0.25 h after the cocaine treatment. Blood samples were taken from the heart 0 and 6 h after death. Each bar represents the mean of 5 animals + S.D.*

TABLE 2—*Ratios of cocaine and cocaethylene concentrations in blood and tissues at each time after death to those at death in rats sacrificed 1 h after treatment with 20 mg/kg cocaine intramuscularly and 2 g/kg ethanol orally.*

Samples	Drugs	Concentration ratios 0	1	3	6 h
Blood*	Cocaine	1.00	—	—	1.10±0.10
	Cocaethylene	1.00	—	—	1.09±0.09
Liver	Cocaine	1.00	0.84±0.03**	0.68±0.07**	0.67±0.02**
	Cocaethylene	—***	—	—	—
Brain	Cocaine	1.00	1.03±0.12	0.94±0.03	0.94±0.10
	Cocaethylene	1.00	1.09±0.07	1.09±0.06	1.19±0.11
Muscle	Cocaine	1.00	1.09±0.06	0.97±0.04	1.04±0.11
	Cocaethylene	1.00	1.04±0.04	1.14±0.10	1.19±0.23

Each value represents the mean of 5 animals ± S.D.

*:Blood samples were taken 0 and 6 h after death.

**:Statistically significant compared with the value at death (Student's t test, $P < 0.05$).

***:The concentration ratios were not calculated because no cocaethylene was detected at death. The lower detection limit for cocaethylene in the liver was about 30 ng/g.

even with cocaine present. The magnitude of cocaethylene formation in postmortem liver may greatly depend not only on liver concentrations of cocaine and ethanol but also on temperature of the body. The liver does not appear to be a good specimen for analyzing cocaine or cocaethylene.

The forensic pathologist and toxicologist should be aware that concentrations of cocaine and cocaethylene in the liver and blood can change significantly postmortem, even during the early stages.

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