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Postmortem Stability of Cocaine and Cocaethylene in Blood and Tissues of Humans and Rabbits

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ABSTRACT: A study was conducted to examine the postmortem stability of cocaine and cocaethylene in rabbit blood and tissues, and to determine whether cocaethylene is produced in decomposed human specimens containing cocaine and endogenous ethanol. Heart blood, liver, brain and femoral muscle taken from rabbits 20 min after oral administration of 20 mg/kg cocaine together with 2 g/kg ethanol were kept at 20–25°C for 5 days. Cocaine and cocaethylene concentrations were in the order brain>liver>muscle>blood, and showed very large intersubject variations at the time of death. Cocaine was degraded rapidly in the blood and liver. However, 12.0 ± 8.5% and 26.2 ± 19.4% of the original cocaine was still detectable in the brain and muscle, respectively. Cocaethylene was degraded more slowly than cocaine in all of the specimens. The pH of the blood remained around 7.4 during a 5-day period; all the other specimens showed pH values of 6.2–6.7 on and after the first day postmortem. When 10,000 ng/g cocaine was incubated with decomposed human blood, liver, brain and muscle homogenates containing 0.29–0.60 mg/g endogenous ethanol at 20–25°C and 37°C, no change in cocaine concentration was observed during the study period of 24 h, and no cocaethylene was detected. The pH values of the homogenates were within the range 4.2 to 5.2 at the beginning of the experiment. It was found that: 1) cocaethylene was more stable in postmortem specimens than cocaine; 2) muscle as well as brain was specimen of choice for detecting cocaine and cocaethylene postmortem; 3) cocaine was resistant to decomposition under acidic conditions; and 4) putrefactive bacteria had no ability to produce cocaethylene even in the presence of cocaine and endogenous ethanol.

KEYWORDS: forensic science, forensic toxicology, toxicology, cocaine, cocaethylene, ethyl alcohol, postmortem stability of cocaine, drug-alcohol interactions

Cocaine abuse is a serious worldwide problem, and the incidence of acute death associated with cocaine use is still high (1). To determine the toxicity of cocaine in corpses, heart blood is usually examined for its cocaine level because an adequate volume of blood can usually be obtained. However, Hearn et al. (2) have reported that blood cocaine levels vary tremendously according to site. This might be due partly to redistribution of cocaine from tissues to the blood (3), a process possibly accelerated by acid conditions (4). Moreover, cocaine in the blood can be degraded rapidly to ecgonine methyl ester by remaining plasma pseudocholinesterase activity depending on the postmortem interval and spontaneously to benzoylecgonine under alkaline conditions (5,6). Thus, the blood cocaine level detected in corpses may not represent level that was present at death. However, Manhoff et al. (7) have documented that cocaine is still detectable even in substantially decomposed bodies.

In cocaine abusers who also drink, cocaethylene, an active metabolite of cocaine, can be formed by a hepatic esterase that metabolizes cocaine to benzoylecgonine in the absence of ethanol (8). Animal experiments have demonstrated that cocaethylene is equipotent to cocaine in producing hepatotoxicity and has a much higher lethality than cocaine (9,10). Although the full clinical importance of cocaethylene is not yet clear, cocaethylene is thought to be responsible, in part, for the prolonged cocaine high associated with drinking in cocaine users because of its longer half-life (11). Thus, both cocaine and cocaethylene should be analyzed for appropriate interpretation of toxicity in decedents (12). Despite growing knowledge about the stability of cocaine in postmortem specimens (5–7,13–17), there is little literature available on the stability of cocaethylene in postmortem blood and tissues.

The purpose of this study was to clarify which tissues are suitable for detecting cocaine and cocaethylene in corpses and whether cocaethylene can be formed in decomposed specimens containing cocaine and endogenous ethanol produced by putrefactive bacteria.

Materials and Methods

Apparatus

A Shimadzu GC-14B (Kyoto, Japan) equipped with a TC-1 capillary column [dimethyl silicone, 15 m × 0.53 mm I.D., 1.0 μm film thickness (GL Science, Tokyo, Japan)] and flame-thermoionic detector (FTD) was used for quantitation of cocaine and cocaethylene. The temperatures of the injection port and detector were 250 and 280°C respectively. The column temperature was programmed to maintain an initial temperature of 150°C for 2 min and then to increase to a final temperature of 280°C at a rate of 10°C/min. The carrier gas was nitrogen at a flow pressure of 15 kPa.

Reagents

Cocaine hydrochloride was purchased from Takeda Chemical Industries (Osaka, Japan). Benzoylecgonine and cocaethylene were prepared from cocaine hydrochloride using published procedures (18–20). All the other chemicals were of analytical grade.

Animal Experiment

Male white rabbits each weighing about 3.5 kg were given a 10 mL/kg aqueous solution containing 0.224% w/v cocaine hydro-

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chloride (0.2% w/v as cocaine base) and 20% w/v ethanol into the stomach, and blood was drawn from the heart 20 min later. The rabbits were then killed by air embolism. The liver, brain and femoral muscle were removed immediately and cooled on ice. The experiment was started using the blood and tissues left separately in glass bottles with caps at 20–25°C. Cocaine and cocaethylene were analyzed at the start of the experiment, and 1, 3 and 5 days later. A portion of each tissue was homogenized in 3 portions of distilled water before analysis. The pH of each specimen was also monitored.

In Vitro Experiment Using Human Blood and Tissues

Drug-free blood, liver, brain and femoral muscle were obtained from an autopsy case. The blood was homogenized in an equal amount of 10% glucose solution, and a portion of each tissue was homogenized in 4 portions of 25% glucose solution. Each homogenate was allowed to decompose at 37°C for 24 h. To the decomposed homogenates was added cocaine hydrochloride at a final concentration of 11,200 ng/g (10,000 ng/g as cocaine base). An aliquot of the mixture was left in a glass bottle with a cap at 20–25°C or 37°C, and analyzed for cocaine and cocaethylene at 0, 3, 6 and 24 h. The pH of each homogenate was also monitored.

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

Two milliliters of blood or 2 g homogenate was mixed with 10 μL 100 μg/mL carbinoxamine maleate 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 2500 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 2500 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-ethylbutane/toluene/isoamyl alcohol (94/5/1 v/v/v) for 30 s using the vortex mixer, centrifuged at 2500 rpm for 5 min, and then this organic phase was evaporated to dryness at 50°C using a gentle stream of air. The residues were reconstituted with 50 μL methanol and a 1- μL aliquot was injected into the GC. Lower detection limits for cocaine and cocaethylene were 10 ng/mL in the blood and 40 ng/g in tissues.

Results

Animal Experiment

As shown in Table 1, cocaine and cocaethylene concentrations with very large intersubject variations were much higher in the brain than in the other specimens 20 min after oral administration of 20 mg/kg cocaine and 2 g/kg ethanol. The blood concentrations of cocaine and cocaethylene at 20 min were as low as 33 ± 15 and 90 ± 4 ng/mL respectively. Cocaine in separated blood became undetectable within 1 day, and the blood cocaethylene within 3 days. Most of the cocaine was degraded in separated liver within 1 day, although 17.2 ± 4.4% of the original cocaethylene remained even 5 days after death. In the brain and muscle, 12.0 ± 8.5% and 26.2 ± 19.4% of the original cocaine was still detectable, respectively, on the fifth day postmortem. Much larger quantities of cocaethylene than cocaine remained in the brain and muscle: 40.7 ± 8.0% and 48.4 ± 7.6% of the original cocaethylene, respectively (Fig. 1). The pH of blood scarcely changed during the 5-day period, remaining around 7.4. In the liver and brain, the pH decreased to below 7.0 on the first day postmortem and then remained almost constant at 6.2 and 6.7 respectively. Muscle pH was 6.8 at the start of the experiment and decreased to around 6.3 one day later (Fig. 2). The mean concentration of blood ethanol was 2.05 ± 0.39 mg/mL at death.

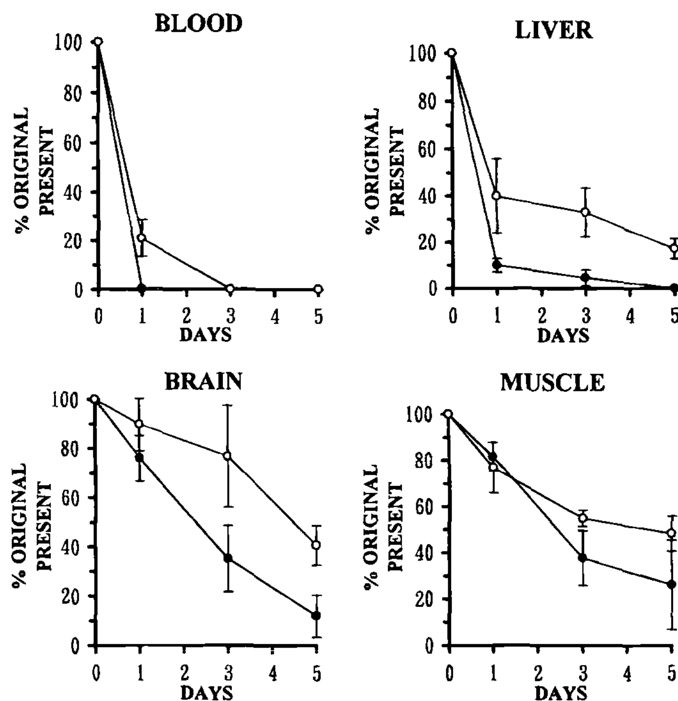

FIG. 1—*Percentage changes in cocaine (●) and cocaethylene (○) concentrations in rabbit blood and tissues stored at 20–25°C. Twenty mg/kg cocaine and 2 g/kg ethanol were given into the stomach simultaneously. Rabbits were sacrificed 20 min after administration. Each point represents the mean of 3 animals ± S.D. The mean concentration of blood ethanol was 2.05 ± 0.39 mg/mL.*

In Vitro Experiment Using Human Blood and Tissues

As shown in Table 2, 0.29–0.60 mg/g endogenous ethanol was detected in the decomposed homogenates of blood and tissues from a man free of drinking and drug use at the start of the experiment. The pH values were within the range 4.2 to 5.2 (Table 2). No change in the concentrations of cocaine added was observed during a 24-h period at 20–25°C and 37°C in any of the decomposed homogenates (Fig. 3). No cocaethylene was detected during the experiment.

Discussion

It has been well documented that cocaine in urine is stable chemically at a pH of less than 7.0 but degraded spontaneously to benzoylecgonine under alkaline conditions (2,16). Moreover, cocaine is metabolized in the blood to ecgonine methyl ester after death even in acidic conditions by residual pseudocholinesterase activity (3).

When cocaine is consumed together with alcoholic beverages, cocaethylene, an active metabolite of cocaine, can be formed in the presence of cocaine and ethanol by hepatic esterases (8). Mouse kidney also has the ability to form cocaethylene (21). Cocaethylene

TABLE 1—*Changes in the concentrations of cocaine and cocaethylene in rabbit blood and tissues stored at 20–25°C.*

Specimen		Concentration (ng/mL or ng/g) 0	1st	3rd	5th day
Blood	Cocaine	33 ± 15	N. D.	N. D.	N. D.
	Cocaethylene	90 ± 4	19 ± 7	N. D.	N. D.
Liver	Cocaine	902 ± 178	93 ± 43	41 ± 29	N. D.
	Cocaethylene	455 ± 128	182 ± 79	153 ± 71	84 ± 45
Brain	Cocaine	2090 ± 1660	1640 ± 1450	953 ± 964	371 ± 331
	Cocaethylene	5920 ± 5790	4920 ± 4420	4180 ± 3430	2420 ± 2180
Muscle	Cocaine	510 ± 609	393 ± 466	262 ± 339	225 ± 289
	Cocaethylene	707 ± 437	497 ± 287	396 ± 243	337 ± 206

NOTE:—Twenty mg/kg cocaine and 2 g/kg ethanol were given into the stomach simultaneously.
Rabbits were sacrificed 20 min after administration.
Each value represents the mean of 3 animals ± S.D.
The mean concentration of blood ethanol was 2.05 ± 0.39 mg/mL.

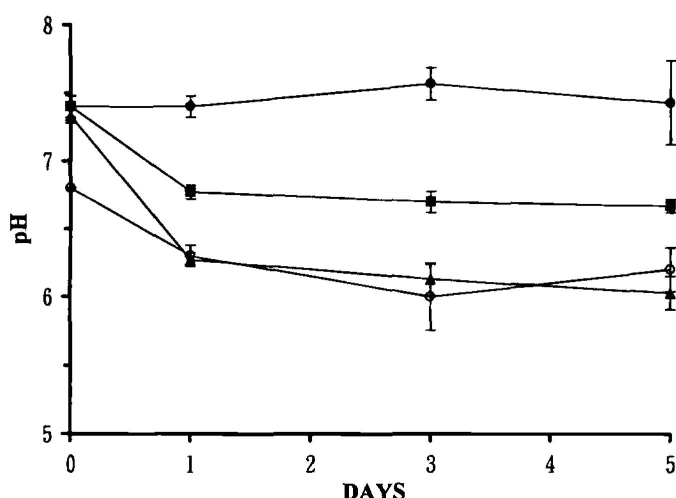

FIG. 2—*Changes in pH values of rabbit blood (●), liver (▲), brain (■), and muscle (○) stored at 20–25°C. Each point represents the mean of 3 animals ± S.D.*

TABLE 2—*Concentrations of endogenous ethanol in decomposed homogenates of human blood and tissues containing 5% glucose, and pH values of the homogenates.*

Homogenate	Ethanol (mg/g)	pH
Blood	0.33	5.2
Liver	0.39	4.5
Brain	0.29	4.3
Muscle	0.60	4.2

NOTE:—The blood was homogenized in an equal amount of 10% glucose.
A portion of each tissue was homogenized in 4 portions of 25% glucose.
Each homogenate was left at 37°C for 24 h.

is just as effective at blocking dopamine reuptake as cocaine in rats and produces the same behavioral alterations as cocaine in rhesus monkeys (22). Moreover, cocaethylene is equipotent to cocaine in producing hepatotoxicity and has a much higher lethality than cocaine in mice (9,10). Although the full clinical importance of cocaethylene is not yet clear, it is thought to be responsible, in part, for the prolonged cocaine high in cocaine users who also drink, because of its longer half-life (11). Thus, both cocaine and cocaethylene should be analyzed for appropriate interpretation of toxicity in decedents (12). However, there is little information available on the postmortem stability of cocaethylene.

In the present study using rabbits treated with cocaine and ethanol, the blood concentrations of cocaine and cocaethylene were very low at death; the liver levels of the substances not as high as the brain levels. This may have been due to poor deposition of cocaine and cocaethylene in the liver and to their rapid distribution from the blood to the brain and muscle (23). Both cocaine and cocaethylene were degraded much faster in the separated blood and liver than in the separated brain and muscle. Although rabbit blood has a very low level of pseudocholinesterase activity (for example, only one fifty-fourth level of human plasma pseudocholinesterase activity (24)), portions of cocaine and cocaethylene in the separated blood may have been metabolized by residual pseudocholinesterase activity because the blood and tissues were left at a room temperature of 20–25°C. Moreover, spontaneous degradation may have accelerated the disappearance of blood cocaine because of the constant blood pH of around 7.4 (2,3). As the blood was taken from the rabbit heart immediately before death, its pH would have been physiologically normal. However, if it had been taken after death, the pH would have been below 7.0 (4). In other tissues, spontaneous degradation can be ignored because the pH of these specimens decreased rapidly to less than 7.0 after death. Thus, the degradation of cocaine and cocaethylene in the liver, brain and muscle may be due mainly to residual esterase activity. The liver, with much larger quantities of esterases, may have facilitated faster postmortem metabolism of cocaine and cocaethylene than brain and muscle. In a previous study using rats, we demonstrated that cocaethylene can still be produced at an early stage postmortem in the liver in the presence of cocaine and ethanol. Formation of cocaethylene, however, was not observed after bodies had been cooled to ambient temperature (25). Postmortem synthesis of cocaethylene in the liver can be ignored in the present study because the blood and tissues were left at the room temperature of 20–25°C. Thus, the slower disappearance of cocaethylene than cocaine in the liver may be due simply to the difference in postmortem stability between cocaine and cocaethylene. Cocaethylene seems to be more stable than cocaine in postmortem specimens. This might reflect a longer half-life of cocaethylene in living individuals than that of cocaine (11). Spiehler and Reed (26) reported that cocaine was evenly distributed throughout human brain and that cocaine in the brain was stable for up to 3 months at −4°C and for 30 days at 10°C. Moreover, Mulé et al. (27) demonstrated that the duration of pharmacological action of cocaine in rats depended greatly on the brain level of

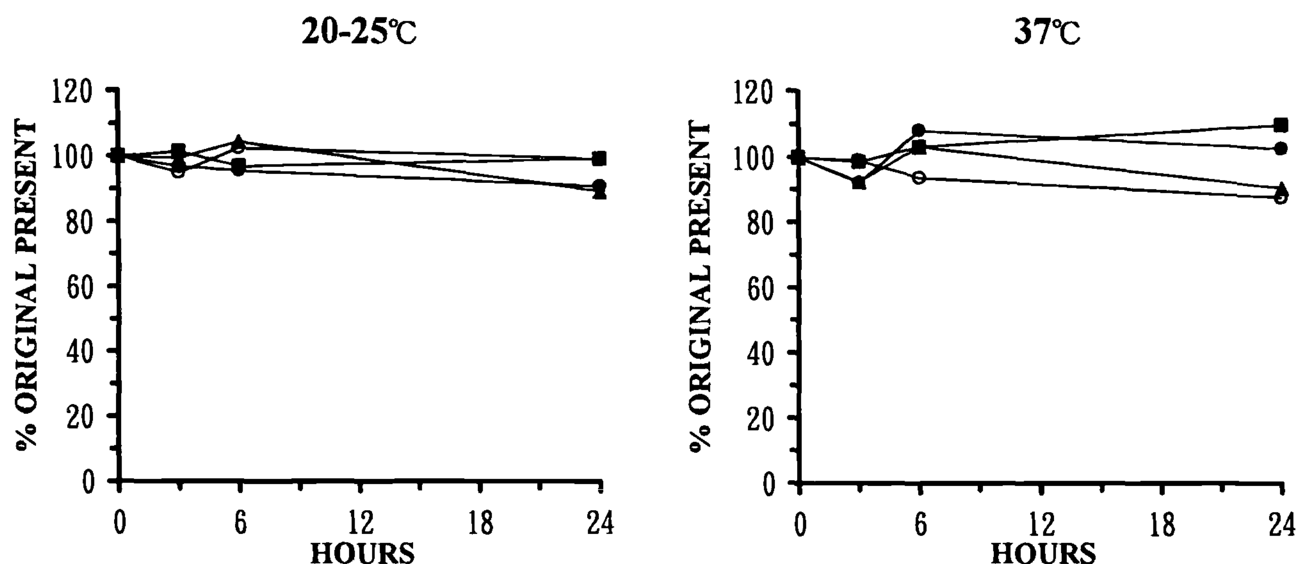

FIG. 3—*Stability of cocaine in decomposed homogenates of human blood (●), liver (▲), brain (■), and muscle (○) containing 5% glucose at 20–25°C and 37°C. The blood was homogenized in an equal amount of 10% glucose. A portion of each tissue was homogenized in 4 portions of 25% glucose. To each homogenate that was left at 37°C for 24 h, cocaine was added at a final concentration of 10,000 ng/g.*

cocaine. Thus, the brain has been thought to be a better sample for postmortem cocaine determination than the blood or liver. The present results suggest that the muscle as well as the brain is specimen of choice when testing for cocaine and cocaethylene after death.

In decomposed bodies, large quantities of ethanol can be produced endogenously by putrefactive bacteria (28). It is still unknown whether cocaethylene is produced in decomposed corpses containing cocaine and endogenous ethanol by bacterial enzymes. The decomposed homogenates of human blood and tissues containing 10,000 ng/g cocaine and 5% glucose showed very low pH values of 4.2–5.2 and relatively large quantities of ethanol, 0.29–0.60 mg/g, were present. In this experiment, we added glucose as a material for ethanol production by putrefactive bacteria (29). No degradation of cocaine in the homogenates was observed, irrespective of temperature, and this resulted in lack of cocaethylene accumulation. The results of the *in vitro* experiment indicate that cocaine is very stable even in decomposed specimens if the pH is kept below 7.0, and that cocaethylene is never produced by putrefactive bacteria even in the presence of cocaine and endogenous ethanol. In addition, the pH values of blood or bloody fluids obtained from nondecomposed and heavily decomposed corpses were within the ranges 6.2–6.8 (mean: 6.58 ± 0.24, n = 5) and 6.4–6.8 (mean: 6.62 ± 0.13, n = 5) respectively. Thus, the reported stability of cocaine even in heavily decomposed bodies (7) is due mainly to acidic conditions within the corpses.

In conclusion, the present study has clarified that 1) cocaethylene is more stable in postmortem specimens than cocaine, 2) muscle as well as brain is specimen of choice for detecting cocaine and cocaethylene in decomposed bodies, 3) cocaine is resistant to decomposition under acidic conditions, and 4) putrefactive bacteria have no ability to produce cocaethylene even in the presence of cocaine and endogenous ethanol.

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