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Deposition of Cocaine in Tissue After Lethal and Repeated Sublethal Administration to Sheep*

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Summary. The deposition of cocaine in tissue after lethal administration to sheep was investigated. In addition, the presence of cocaine in tissue obtained from sheep treated for 30 days with a sublethal dose (2.4 mg/kg b.wt.) after 1-day, 1-week, or 1-month withdrawal was studied. The determination of cocaine was performed by radioimmunoassay. The concentrations measured represent the sum of cocaine and its metabolites. The presence of cocaine was also qualitatively proven by gas chromatography/mass spectrometry. After a lethal dose administration cocaine was found in all investigated organs. The highest concentrations were present in liver, bile, and kidney. In tissue obtained from the sheep treated daily with a sublethal dose and killed after 1-week withdrawal, the concentrations found were significantly lower. After 1-month withdrawal, cocaine was not to be discovered in tissue.

Key words: Cocaine in tissue – Intoxication, cocaine

Zusammenfassung. Die Ablagerung von Kokain in Schafgewebe nach letaler Dosis wurde untersucht. Zusätzlich wurden die Kokainkonzentrationen in Gewebeproben von Schafen, die 30 Tage lang mit je 2,4 mg/kg KG Kokain und einschließlich 1 Tag, 1 Woche bzw. 1 Monat danach ohne Kokain behandelt wurden, bestimmt. Die Bestimmungen erfolgten mittels Radioimmunoassay. Die gemessenen Konzentrationen stellen die Summe von Kokain und Metaboliten dar. Zusätzlich wurde mittels Gas-Chromatographie/Massen-Spektrometrie das Vorhandensein von Kokain qualitativ nachgewiesen. Nach tödlicher Kokaindosis wurde die Droge in allen untersuchten Organen gefunden. Die höchsten Konzentrationen wurden in Leber, Galle und Niere gemessen. Die Konzentrationen im Gewebe von Schafen, die 30 Tage mit Kokain und danach 1 Woche ohne Kokain behandelt wurden, waren wesentlich niedriger. Keine meßbaren Konzentrationen konnten nach 1 Monat ohne Kokaingabe nachgewiesen werden.

Schlüsselwörter: Kokain, Verteilung im Gewebe – Vergiftung, Kokain

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Introduction

Cocaine is almost certainly one of the drugs in longest use. The drug increased the capacity for muscular work, alleviated hunger and fatigue, and is anesthetic. These properties explain the practice of chewing coca leaves also today by the South American Indians.

Owing to its euphoric, sexually stimulating, and aphrodisiac properties, cocaine was already used in the bohemian society of 1914. However, in the last several years a worldwide explosion of cocaine abuse has been observed. An increase in cocaine-related deaths has been reported.

In this study, we have investigated the tissue deposition of cocaine after administration of 40 mg cocaine/kg b.wt. In previous investigations we already observed that this is a lethal dose for sheep. This observation is approximately in accordance with reports for humans. Observations for the lethal dose of cocaine in sheep are not available. Moreover, the presence of cocaine in tissue from sheep treated daily for up to 1 month with cocaine after 1-day, 1-week, or 1-month withdrawal was studied.

Materials and Methods

To eliminate the stress induced by daily i.v. application of cocaine before the experiments a permanent catheter was inserted under anesthesia into the arteria cervicalis superficialis in each sheep. The i.a. catheter is more suitable for long-time investigations than the i.v. catheter because the risk of obdurateness by sclerosis is lower, and the fixation is also better. The artery was infused during the experiments with heparinized saline (2 ml/h, 10 IU/ml). On postoperative day 3 cocaine hydrochloride (4 mg/kg) was administered i.a. at 15-min intervals in three merino sheep, 30–40 kg b.wt. (group 1). After application of 1.6 g cocaine the sheep were killed, the organs removed, and 1 g of each organ homogenized with 5 ml normal saline. The homogenate extracts were centrifuged (1,000 × *g* for 10 min), and the supernatants were used directly for assay.

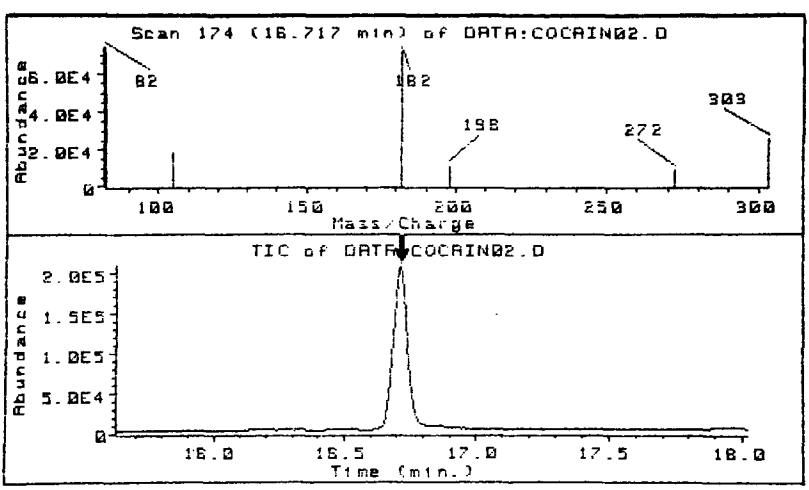
In four sheep a sublethal cocaine hydrochloride dose (2.4 mg/kg b.wt.) was administered daily for up to 30 days. One sheep was killed on day 31 after start of the experiments (group 2), two sheep after 1-week withdrawal (group 3), and one sheep after 1-month withdrawal (group 4), respectively. The organs were removed and the samples prepared as described above.

The cocaine concentrations were determined by radioimmunoassay according to the method described previously (Balabanova et al. 1987). The concentrations measured represent the sum of cocaine and its metabolites.

For the determination of cocaine by gas chromatography/ mass spectrometry the samples were homogenized as above, the homogenate extracts were back-extracted with chloroform after alkalinization. This extract was used for solid injection into gas chromatograph/mass spectrometer at 290°C evaporator temperature. Helium was used as carrier gas at a flow rate of 0.9 ml/min. A 30-m bonded phase fused-silica OV-1 capillary column 0.2 mm i.d. was employed. Solid injection at 290°C evaporator temperature and 50°C oven temperature. Following injection, the oven temperature was rapidly increased to 210°C and kept for 2 min, then programmed at a rate of 2°C/min to a final temperature of 220°C and kept for 8 min at this temperature. The ionization mode was electron impact, ionizing voltage; 70 eV. Selected ions; m/z 303 (M^+) and mass fragments: m/z, 272, 198, 182, 105, 82 were monitored repeatedly and stored for subsequent processing in a Hewlett-Packard 9000/300 personal computer. The lowest detection limit of cocaine-HCl under these conditions was 100 fg/injection (Balabanova et al. 1987).

Table 1. Cocaine concentrations found in tissues, bile, and urine of sheep after lethal cocaine injection (group 1) and after daily administration of a sublethal cocaine dose for up to 30 days and 1-day (group 2) or 1-week (group 3) withdrawal

Tissues	Cocaine concentrations (µg/g or µg/ml)		
	Group 1	Group 2	Group 3
Liver	28.00	2.60	0.11
Kidney	8.80	3.35	0.07
Pancreas	2.90	0.45	0.07
Thymus	2.40	0.85	0.02
Lung	1.80	0.60	0.02
Intestinal wall	1.70	0.80	0.01
Heart muscle	1.60	0.45	0.02
Stomach wall	1.50	0.40	0.03
Wall of the bladder	1.50	0.60	0.02
Fatty tissue	1.40	0.40	0.02
Spleen	1.20	1.00	0.02
Testes	1.10	0.40	0.03
Adrenal	0.80	1.00	0.03
Brain	1.00	0.07	0.02
Bile	28.00	1.21	0.07
Urine	16.50	0.00	0.00



STANDARD (5 ng)

Fig. 1. Mass spectrum and chromatogram of cocaine standard (5 ng)

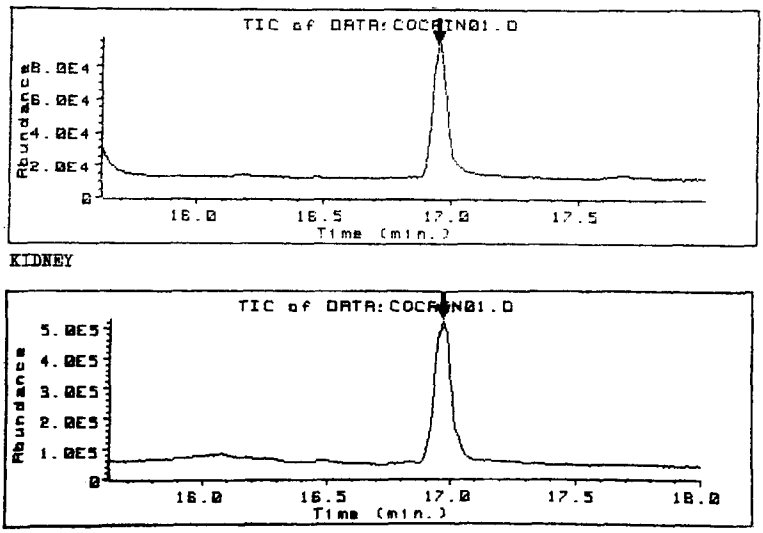


Fig. 2. MS/GC determination of cocaine in tissue of sheep after lethal cocaine injection (group 1)

Results

After lethal injection the highest cocaine concentrations were found in (μg/g, μg/ml mean value): liver 28, bile 28, and kidney 8.8. In the remaining organs the concentrations were from 0.8 μg/g to 3 μg/g. In the organs of sheep killed on day 31 after the start of experiments the highest cocaine values were found also in kidney (3.4 μg/g), liver (2.6 μg/g), and bile (1.2 μg/ml). The concentrations found in the organs of sheep killed after 1-week withdrawal were significantly lower. The highest values were found in the liver (0.11 μg/g), bile (0.075 μg/ml), and kidney (0.072 μg/g). The concentrations are given in Table 1.

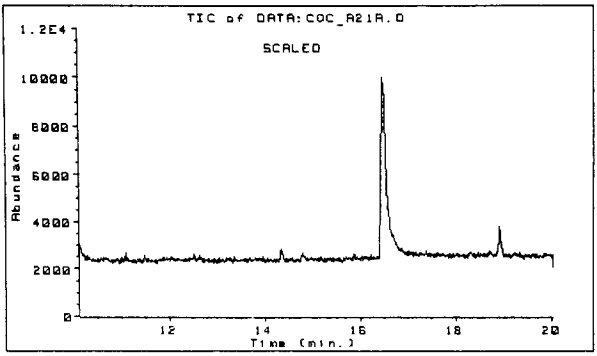
After 1-month withdrawal measurable concentrations in 1-g tissue samples were not found.

Cocaine in bladder urine was found only in the sheep killed after a lethal cocaine dose (16.5 μg/ml). In the remaining groups no cocaine was found in blood and urine.

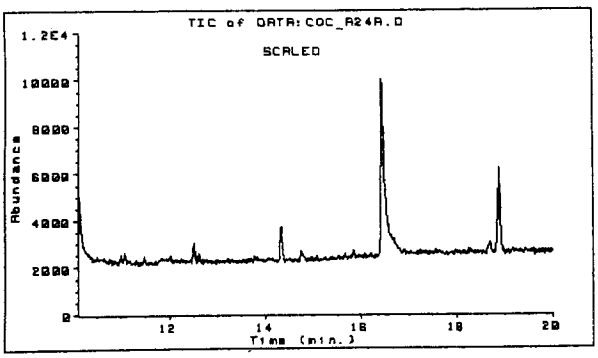
The mass spectrum of cocaine and its mass fragments, as well as those obtained by tissue extract are shown in Figs. 1–3.

Discussion

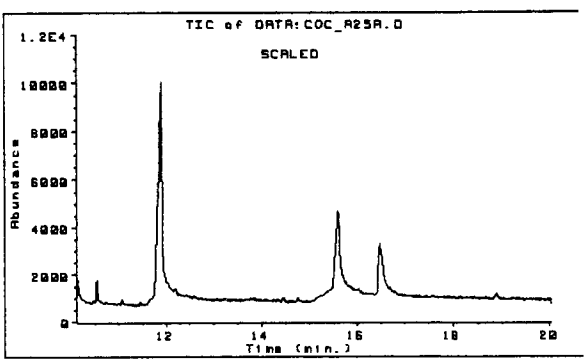
Data of cocaine deposition in fetal overdoses have been described already by Price (1974); Lundberg et al. (1977); DiMaio and Garriott (1978); Bednarczyk et al. (1980); Nakamura and Noguchi (1981); Poklis et al. (1985). However, the concentrations measured are highly controversial. Thus, Poklis et al. (1986) found the highest values in kidney, spleen, and bile. DiMaio and Garriott



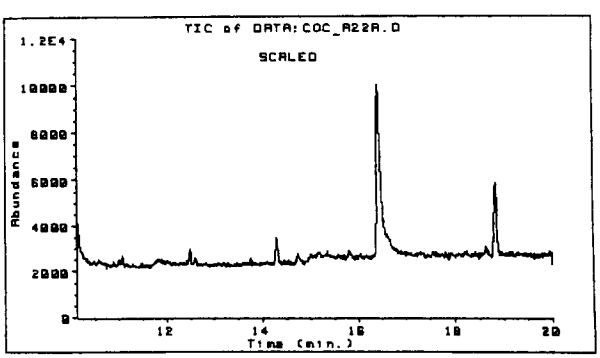
KIDNEY



LIVER



BILE



STOMACH

Fig. 3. MS/GC determination of cocaine in tissue of sheep after daily administration of cocaine (30 days) and 1-week withdrawal (group 3)

(1978) found the highest level in one case in the bile, in one case the value in bile was very low, and in two cases not available. The cocaine concentrations found in the liver were significantly lower than those reported by Poklis (1986), and in one case also not available. In the report by Price (1974) spleen concentrations exceeded those of the kidney. The difference between the concentrations measured may be due to differences between dosing, time of death, and the hepatic biotransformation. The last was related to the liver condition.

In this study, the highest concentrations after lethal doses were found in the liver and bile. In experiments with dogs, Woods et al. (1951) found the highest cocaine levels in kidney and spleen. The difference between the results of Woods, the above mentioned findings in man, and our experiments with sheep may be explained by the different biotransformation rates in man and animals.

Cocaine is disposed very rapidly in the body. However, the drug is also rapidly excreted and degraded as indicated by the low concentrations found after 1-week withdrawal. Our study indicated that immediately after fatal cocaine abuse, the drug is disposed predominantly in liver and bile. After 7 days without cocaine, the highest concentrations are present in kidney and liver.

In the body cocaine is metabolized to benzoylecgonine, ecgonine methyl ester, ecgonine, and norcocaine. The radioimmunologic method used in our study determined the sum of cocaine and its metabolites. On the contrary, the gas chromatographic/mass spectrometric determination is cocaine-specific. The method proved that cocaine, at least in part, remained unchanged in the body also after 1-week withdrawal.

In conclusion, the significant differences in cocaine concentrations found in the organs of the four animal groups make it possible to determine approximately the time and dosis of cocaine abuse after death.

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