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A Rapid Method for the Determination of Cocaine in Brain Tissue

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ABSTRACT: A rapid procedure is described for the extraction and analysis of brain samples for cocaine and benzoylecgonine. Human brain tissue was sectioned at autopsy, and samples were subjected to a lipase digestion, subsequent to solid-phase extraction. The distribution of cocaine and benzoylecgonine throughout different regions of the brain was determined by high-performance liquid chromatography.

KEYWORDS: toxicology, cocaine, chromatographic analysis, solid-phase extraction, high-performance liquid chromatography

The ever-increasing drug abuse problem has resulted in cocaine being frequently encountered in postmortem samples, either as the direct cause of death or as a contributing factor. Cocaine is unstable in blood or aqueous solutions [*1*] but has been found to be stable in frozen brain tissue on reanalysis after three months [*2*]. It has been suggested that brain tissue is a better sampling site than blood for the determination of cocaine concentration. Numerous methods have been described for the analysis of cocaine and its hydrolysis product, benzoylecgonine, using high-performance liquid chromatography (HPLC) [*3*] and gas chromatography/mass spectrometry (GC-MS) [*4,5*]. All of these methods require that the drugs be first extracted from the biological matrix. Drug extraction from tissue homogenates at present, utilizes liquid-liquid extraction of the drug prior to analysis. However, the use of bonded silica columns (solid phase) has gained popularity in many areas as an alternative, as it offers the advantages of avoiding emulsion formation and minimizing the volumes of solvent required and is, above all, more rapid than traditional extraction procedures [*6,7*].

The authors of this paper have developed a method for the rapid extraction of homogenized brain tissue using silica-bonded C2 columns after lipid digestion to break down fatty material. The physical nature of the sorbents allows the homogenate to pass through the column. This extraction process was used for the determination of cocaine and benzoylecgonine in postmortem samples using HPLC analysis.

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Materials and Methods

Materials

Cocaine hydrochloride, benzoylecgonine, and bupivacaine were obtained from Sigma, as was triacylglycerol lipase. Tris(hydroxymethyl)aminomethane (Tris) was obtained from Bio Rad Laboratories. Postmortem brain tissue, removed at autopsy, was sectioned and stored at −20°C until required for analysis.

Extraction Procedure

One-gram samples of brain tissue were homogenized with 2 mL of 0.2*M* Tris buffer adjusted to pH 6.3 with orthophosphoric acid. One milligram of triacylglycerol lipase was then added, together with bupivacaine (0.5 μg/mL) as the internal standard. The samples were then incubated for 2½ h at 50°C. Drug-free brain homogenates were spiked with various concentrations of cocaine prior to enzymatic digestion to ensure that the digestion process had no effect on the stability of the drug.

Bond Elut columns, containing C2 packing material and with a capacity of 3 mL, were positioned in a Vac-Elut system. Vacuum pressure was adjusted to 15 to 20 mm Hg, and each column was activated by washing with 3 × 1 mL of methanol, followed by 3 × 1 mL of molar sodium hydrogen carbonate at pH 8.5. Without allowing the column to dry out, the brain homogenate, together with 1 mL of carbonate buffer, was applied to the column and drawn through under vacuum. The sample was allowed to dry on the column for 30 s before it was washed with 3 × 1 mL of carbonate buffer, followed by 1 mL of 5% methanol in water. The adsorbed drugs were then eluted from the column with 5 × 500 μL of chloroform/isopropanol (4:1). The extracts were evaporated to dryness under a stream of nitrogen at room temperature and reconstituted in 200 μL of HPLC mobile phase for liquid chromatography analysis.

Analytical Procedure

The HPLC method was essentially a modification of that described in a previous article [7]. The system consisted of a Perkin-Elmer Series 3B chromatographic pump, which was used to deliver solvent at a flow rate of 1.5 mL/min. The eluent was monitored at 230 nm with a Waters M-490 multiwavelength programmable UV detector. The column was a 15 cm by 4.5-mm inside diameter (ID) 5-μm C8 (IBM) fitted with a rheodyne injection valve incorporating a 20 μL loop. Separation was achieved with an eluent consisting of 0.025*M* potassium dihydrogen phosphate (adjusted to pH 2.9 with orthophosphoric acid)/acetonitrile/diethylamine (91:7:2). All the solvents used were HPLC grade and were filtered and degassed immediately prior to use. Quantitative analysis was based on peak area calculations relative to the internal standard, and the calibration curves for both cocaine and benzoylecgonine were found to be linear over the range 0.05 to 5 μg/mL. Each measurement was taken as the average of three determinations. The extraction efficiency was estimated by comparison of the peak areas of the extracted drugs with pure standards.

Results

The HPLC system described gave a good separation of cocaine and its metabolite benzoylecgonine (BZE). A chromatogram of an extract of actual postmortem brain tissue is shown in Fig. 1. Cocaine has a retention time of 11.7 min and benzoylecgonine of 6.5 min, with the internal standard eluting after 14 min. No interference due to endogenous

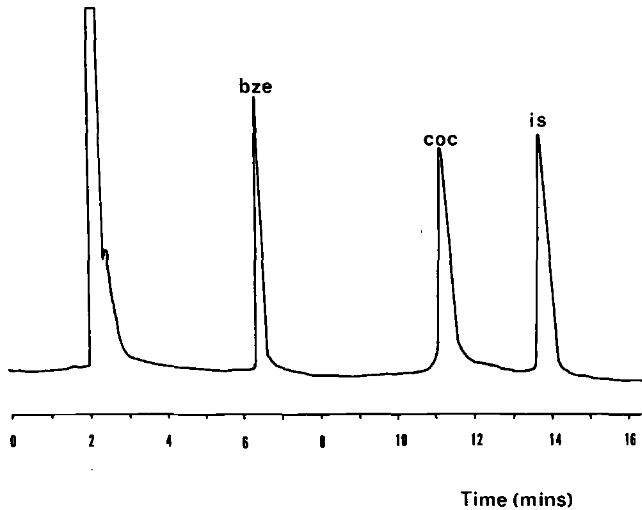

FIG. 1—*HPLC chromatogram of an extract of a postmortem brain tissue sample, showing the presence of approximately 1 μg/g each of cocaine and benzoylecgonine. Bupivacaine is the internal standard.*

materials was seen in brain extracts. The minimum detectable level (signal-to-noise ratio > 2) was 30 ng/mL.

Several different column packing materials were evaluated for the extraction of cocaine and BZE from brain homogenates. While C18 and C8 have previously been found to be suitable for the extraction of these drugs from urine, plasma, and, to some extent, whole blood, these columns were unsuitable for brain homogenates because of blockage of the column. We assume that lipids present in the brain homogenates cause the extraction columns to become blocked as a result of their interaction with the lipophilic packing materials. Enzymatic digestion of the tissue homogenates with lipase, followed by the application of the sample to a more polar C2 allowed the problems of column blockage to be avoided. Cocaine and benzoylecgonine were found to be stable during the process of incubating the drugs at 50°C for 2.5 h, provided the pH of the solution was kept below 7. The recovery of cocaine and benzoylecgonine from brain homogenates spiked with cocaine and benzoylecgonine at a concentration of 1 μg/g; the values were 92 ± 6% for cocaine and 80 ± 5% for benzoylecgonine. Similar recoveries were seen with concentrations of up to 10 μg/g. The procedure was evaluated by the extraction of postmortem brain tissue, and the results of these analyses are represented in Table 1.

In the three cases examined, the mean cocaine blood concentration was 3.7 μg/mL, and the mean concentration in the brain was 32.9 μg/g (range, 0.53 to 91.0 μg/g). The mean blood/brain ratio was 0.168. Case 1 was an 18-year-old male who died as a result of a gunshot wound to the chest. Cases 2 and 3 were a 22-year-old male and a 28-year-old male, respectively, who died as a result of cocaine overdose. No information concerning their drug abuse histories was available.

Discussion

Based on the examination of three human brains in this study, cocaine and benzoylecgonine appear to be distributed throughout the different regions of the organ. This is in agreement with the findings of an earlier study by Spiehler and Reed [2]. Significant differences in the concentration of cocaine were, however, apparent in different regions of the brain. For example, in Case 3, the cocaine concentration in the basal ganglia was

TABLE 1—*Cocaine and benzoylecgonine concentrations, in micrograms per gram.*

Region of the Brain	Case 1		Case 2		Case 3	
	Cocaine	BZE	Cocaine	BZE	Cocaine	BZE
Blood (μg/mL)	(0.10)	(1.15)	(3.00)	(1.82)	(11.1)	(3.6)
Basal ganglia	0.68	0.7	–	–	91.0	2.9
Cerebellum	0.53	0.88	26.80	0.59	34.7	1.36
Cerebral white	–	–	–	–	45.2	2.4
Cerebral grey	–	–	–	–	58.8	2.1
Hypothalamus	0.60	0.35	–	–	41.4	2.0
Hippocampus	1.50	–	–	–	45.6	2.0
Motor cortex	0.53	2.38	–	–	–	–
Frontal cortex	–	–	40.45	1.01	–	–
Lenticular nuclei	–	–	38.40	1.20	–	–
Thalamus	–	–	50.78	0.64	–	–
Occipital cortex	–	–	37.7	0.69	–	–
Pons	–	–	45.1	0.00	43.5	2.6
Temporal lobe	–	–	12.1	2.77	–	–
Medulla	–	–	–	–	42.7	2.4

91.0 μg/g, in comparison with 34.7 μg/g in the cerebellum. The concentrations of cocaine found in the brain were generally four to eight times greater than that found in the blood. The absence of large concentrations of benzoylecgonine supports previous reports of the stability of cocaine in the brain [2,8]. It has been suggested that brain tissue is a better sample than either blood or liver for cocaine determinations. However, the presence in the brain of large concentrations of fats and other endogenous materials which may interfere with the assay have necessitated the use of lengthy extraction procedures. The procedure outlined above is offered as a rapid and efficient alternative method for the extraction of cocaine from brain tissue.

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