Journal of Chromatography, 621 (1993) 89–94
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 7096

Short Communication

Gas chromatographic–mass spectrometric determination of plasma and brain cocaine in mice

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(First received June 15th, 1993; revised manuscript received August 23rd, 1993)

ABSTRACT

A gas chromatographic–mass spectrometric method is described for the determination of cocaine in mouse plasma microsamples and brain. Cocaine and [$^2H_3$]cocaine were extracted with pentane–isopropyl alcohol (97:3, v/v), chromatographed on a (5% phenyl) methylpolysiloxane capillary column, and detected by selected-ion monitoring of electron impact generated m/z 182 and 185 fragment ions. The small sample size (50 μl), simplicity of workup, and high response linearity (mean $r = 0.9993$) distinguish the method. Cocaine was found in mouse brain at approximately 5 times greater concentration than in plasma after 20 or 40 mg/kg subcutaneous doses.

INTRODUCTION

The present analytical method was developed to explore the pharmacokinetics of cocaine in mice. The analysis of cocaine from biological samples requires special consideration of factors contributing to post-sampling drug degradation. Plasma esterase(s) rapidly hydrolyze the benzoate ester group of cocaine (Fig. 1) to yield methylecgonine [1,2]. This process may be inhib-

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0378-4347/93/$06.00

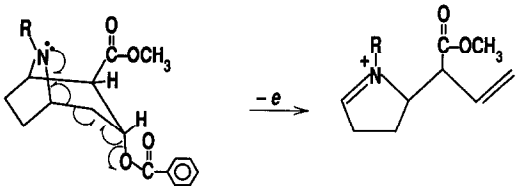

Fig. 1. Structures of cocaine ($R=CH_3$), [$^2H_3$]cocaine ($R=C^2H_3$), and the corresponding EI generated ions selected for monitoring by GC–MS.

ited by the prompt addition of sodium fluoride to the samples [3–6]. The methyl ester in the structure of cocaine, though also subject to a sodium fluoride sensitive enzymatic deesterification [7], is especially prone to base-catalyzed hydrolysis to yield benzoylecgonine [8,9]. This non-enzymatic pathway becomes of potential significance after alkalinization of samples in preparation for extraction. While cocaine hydrolysis may be minimized [10] or prevented [5] by freezing the samples until analysis, the use of deuterated cocaine as an internal standard provides control for any possible drug deesterification during sample workup in addition to potentially improving sensitivity through carrier effects [11].

Gas chromatographic (GC) methods for cocaine bioanalysis which have utilized electron capture [12], flame ionization [13] or nitrogen–phosphorous [14] detection are limited to the use of nondeuterated internal standards and do not exhibit the molecular specificity of mass spectrometric (MS) detection [15]. Of the reported MS methods for cocaine analysis, electron impact (EI) ionization [16–22] provides greater technical simplicity than methods involving chemical ionization [23–26]. Among the EI methods, [$^2H_5$]cocaine (phenyl labeled) [18] and [$^2H_3$]cocaine (N-methyl labeled) [16,20,22] have been incorporated as internal standards, with the use of [$^2H_3$]cocaine permitting greater inherent sensitivity for selected ion monitoring because the deuterium resides in a higher abundance ion. However, these EI methods incorporating deuterated internal standards have not been applied to brain nor plasma samples, and in particular, not to the low microliter plasma volumes frequently encountered in pharmacokinetic studies with small animals.

The capillary GC–MS–EI isotope dilution method for cocaine determination described in the following investigation (1) uses plasma microsamples (50 μl) in addition to brain, (2) avoids multiple step extractions [16,17] and chlorinated organic extractants [17,18,20,22], (3) eliminates packed column GC [16], and (4) provides high response linearity. Application of this method to the plasma–brain distribution of cocaine in mice following subcutaneous administration of 20 and 40 mg/kg doses of the drug is reported.

EXPERIMENTAL

Chemicals

Cocaine hydrochloride (batch No. 6907-1022-167C, purity >95%) and [$^2H_3$]cocaine (batch No. 3995-6-B, purity >98%, isotopic purity: 99.77% [$^2H_3$], 0.23% [$^2H_2$]) were obtained from the National Institute of Drug Abuse (Rockville, MD, USA). Sodium fluoride was from Baker (Phillipsburg, NJ, USA). Sodium borate was from Mallinckrodt (Paris, KY, USA). All solvents were of HPLC grade. Acetonitrile was from Burdick and Jackson (Muskegon, MI, USA). Isopropyl alcohol was from Curtin Matheson (Houston, TX, USA). *n*-Pentane and perchloric acid were from Fisher (Fairlawn, NJ, USA).

Sample collection

Eight male C57BL/6J mice (30–35 g) were administered cocaine hydrochloride subcutaneously in a volume of 0.1 ml/10 g body weight; 4 were dosed at 20 mg/kg and 4 at 40 mg/kg (base). Blood and brain tissues were obtained from one mouse of each group at times 0.5, 1, 2 and 3 h after dosing. Blood was collected from the infraorbital sinus into 4 heparinized capillary tubes (75 μl) just prior to decapitating each mouse. The contents of two capillary tubes were immediately transferred to each of 2 conical glass tubes (2 ml) which contained the oven (70°C) evaporated residue of 45 μl of 1% sodium fluoride (calculated to provide 0.3% blood sodium fluoride). The conical tubes were gently shaken for 5 s, then

centrifuged (Dynac, Clay, Adams, Parsippany, NJ, USA) for 10 min at the maximum setting. The duplicate plasma samples (50 μl) were then transferred to separate screw-cap culture tubes (13 mm × 100 mm) and stored at −70°C. The mouse brains were removed immediately after sacrifice, sectioned along the midline, and stored at −70°C. One plasma sample (50 μl) or one-half brain (0.2–0.25 g) was used for each analysis.

Plasma extraction

Plasma extractions were conducted in the above screw-cap culture tubes. Aqueous sodium fluoride (1%, 250 μl) containing 80 ng of [$^2H_3$]cocaine was added to each tube. The [$^2H_3$]cocaine (used as the free base in methanol, 0.1 μg/μl) was added to the reservoir of aqueous sodium fluoride immediately before use. Calibration standards, run in parallel with the unknowns, utilized aliquots of blank mouse plasma (50 μl). Cocaine hydrochloride in methanol (5 or 50 ng/μl, base) was added to the calibration samples to typically provide 0, 0.04, 0.1, 0.2, 0.4, 1, 2, 3 and 4 μg/ml cocaine (base) concentrations. The standards and the plasma unknowns (50 μl) were then alkalinized to approximately pH 9 by adding saturated aqueous sodium borate (0.75 ml). Pentane–isopropyl alcohol (97:3, v/v), 3 ml, was added to each tube and the samples were extracted by vortex-mixing for 10 s followed by shaking at a 45° angle for 30 min. After centrifugation (2000 *g*) for 15 min, Pasteur pipets were used to transfer the organic phases to disposable screw-cap 4-ml vials. The organic phases were then evaporated to dryness under streams of nitrogen at 25°C, and the vials were capped and stored at −20°C until analysis.

Brain extraction

Brain samples were placed in 15-ml Nalgene centrifuge tubes containing aqueous sodium fluoride (1%, 250 μl). Calibration standards, run in parallel with the unknowns, utilized portions of blank mouse brain (0.2–0.25 g) which were fortified with cocaine hydrochloride in methanol (50 or 200 ng/μl, base) to typically provide 0, 0.4, 1, 2, 4, 8 and 12 μg/g brain cocaine (base) concentrations. Perchloric acid (0.1 *M*, 1 ml) containing 1 μg of [$^2H_3$]cocaine was then added to all samples. The [$^2H_3$]cocaine (used as the free base in methanol, 1 μg/μl) was added to the reservoir of perchloric acid immediately before use. The samples were homogenized (Polytron) for 20 s and centrifuged (3000 *g*) for 20 min. The clear supernatants were then transferred to screw-cap culture tubes (13 mm × 100 mm), avoiding any white solid surface layer. Saturated aqueous sodium borate (1.5 ml) was added to each supernatant to provide a pH of approximately 8.6, then these were extracted according to the method used for alkalinized plasma samples (see above).

Instrumental analysis

All analyses utilized a Finnigan Model 9610 GC-Model 4000 MS interfaced to an IBM-AT computer using a Teknivent Vector/One data system and software (St. Louis, MO, USA). The injector was adapted to capillary bore using a 17.8 cm conversion sleeve and a reducing union (Supelco, Bellefonte, PA, USA). The MS was calibrated with perfluorotributylamine (FC-43) using the *m/z* 219 and 220 ions underresolved to gain sensitivity. Detection was by selected ion monitoring with EI ionization (Fig. 1) at 60–70 eV and 280–300 μA. The electron multiplier was operated at 1825–1925 V. The data system acquired two channels of ion current: that of the cocaine fragment [27] *m/z* 182 (59% relative abundance) and that of the corresponding trideuterated fragment *m/z* 185 (56% relative abundance). The data system scan rate was every 0.1 s with a sweep width of 0.1 a.m.u., integrating each acquisition sample for 4 ms.

Each sample was reconstituted with acetonitrile (20 μl) immediately before injection. This volume was reduced under a stream of nitrogen to approximately 4 μl for the lowest 2 plasma calibration standards. A Hamilton 0.5-μl syringe was used to inject 0.1 μl by the splitless mode onto a (5% phenyl)methylpolysiloxane fused silica column, 30 m × 0.32 mm I.D., 0.25 μm film thickness (DB-5, J & W Scientific, Folsom, CA, USA). The filament was powered 1.75 min after sample injection. The column oven was main-

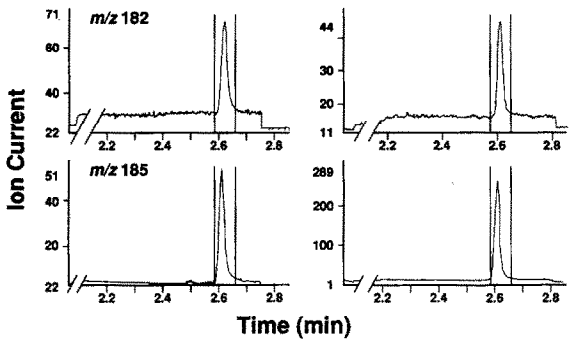

Fig. 2. Selected-ion chromatograms from 0.1 μg/ml cocaine (upper left) extracted from 50 μl mouse plasma and 1 μg/g of cocaine extracted from 0.2 g mouse brain (upper right). Lower ion profiles are those for the associated internal standard, [$^2H_3$]cocaine. The vertical lines flanking the chromatographic peaks indicate the boundaries used for peak-area integration.

tained at 240°C and the injector port and interface oven at 250°C. The helium carrier gas linear velocity was 50 cm/s. Under these conditions, cocaine and [$^2H_3$]cocaine eluted 2.60 and 2.53 min after injection, respectively (Fig. 2).

Calculations

Cocaine concentrations in pharmacokinetic samples and plasma recovery samples were calculated from the slope and intercept of the associated standard curve, plotted as GC peak-area ratio (cocaine/[$^2H_3$]cocaine) *versus* known cocaine concentration. The plasma recovery of cocaine was established by adding cocaine (20 ng) to the final organic extracts of duplicate blank (but internally standardized) samples run in parallel with a set of calibration standards, then comparing the theoretical concentration with the two values found. The cocaine recovery from mouse brain was determined by adding cocaine (1 μg) to the first 2 of 4 blank brain samples, then extracting all four samples as above. Cocaine (1 μg) was then added to the final organic extracts of the remaining two blank samples. The mean peak-area ratio found for these latter two samples was taken as 100% recovery and compared against the peak-area ratios of the first two samples.

The accuracy and precision of the present method was assessed by back-calculating individual standard data points using the slope and intercept of the associated standard curves from 4 separate runs.

RESULTS AND DISCUSSION

Analytical method

Blood was collected from the supraorbital sinus of the mice rather than as trunk blood. This avoided possible mixing of the blood with stomach contents where cocaine may be in high concentration due to ion trapping [28]. However, sinus blood sampling does limit the practical volume of plasma to the duplicate 50-μl samples collected in this study. The amount of sodium fluoride added to the centrifuge tubes used to separate the plasma was calculated to provide a plasma sodium fluoride concentration of 0.3%. This percentage is high enough to effectively inhibit esterase activity [6,7], yet below a concentration associated with a pronounced salting out effect [29,30] which may potentially alter the partitioning of cocaine between the plasma and red cells.

The recovery values for cocaine extracted from the duplicate plasma samples were 77 and 79% and the recovery values for the brain samples were 56 and 61%. Nonsilanized extraction tubes were used since silanization did not improve recovery. Alkalinization of the samples with the borate solution permitted organic phase extraction of cocaine and [$^2H_3$]cocaine while avoiding an extreme of pH. Pentane was selected as the organic extractant due to the ease of evaporation and low relative toxicity. Isopropyl alcohol was added to the pentane to limit sample emulsion formation and to reduce adsorptive losses. No back extraction nor acidified sample washing step was found necessary to provide chromatograms free of extraneous peaks (Fig. 2). Methylecgonine, which generates an m/z 182 ion [27] in common with cocaine, did not interfere with the detection of cocaine since this metabolite eluted prior to powering the filament. In spite of the analytical advantages of GC temperature programming, an isothermal mode was used in the present study to increase sample throughput.

TABLE I

ACCURACY AND PRECISION

Plasma concentration of cocaine (μg/ml)		n	Coefficient of variation (%)
Added	Detected (mean)[a]		
0.04	0.04	4	20.7
0.2	0.23	4	14.7
0.4	0.42	4	6.5
1.0	0.94	4	5.5
2.0	2.00	4	3.3

[a] Evaluated by back-calculating individual standard data points using the associated calibration plot from four separate runs.

Samples were typically injected into the GC every 4 min which, when combined with the ease of sample extraction, provided for an overall rapid analytical method.

The quality of the cocaine determinations is presented in Table I. Precision improved from 20.7 to 3.3% (coefficient of variation) in progressing from 0.04 to 2.0 μg/ml cocaine plasma calibration standards. The nine calibration plots generated in the course of validating and applying the present method to plasma and brain samples provided high mean response linearity (r = 0.9993). The lowest calibration standard, 40 ng/ml cocaine using 50 μl of plasma, provided a signal-to-noise ratio of approximately 6. The 0 ng/ml cocaine calibration samples generated cocaine/[$^2H_3$]cocaine peak-area ratio values of 0.007–0.037 due to the low m/z 182 ion current produced by [$^2H_3$]cocaine under the instrumental tuning and resolution conditions.

Application

Approximately fivefold greater concentration of cocaine was found in mouse brain than in plasma over the 3-h period following the subcutaneous administrations of either the 20 or 40 mg/kg dose (Fig. 3). The highest cocaine concentration in both matrices occurred 1 h after administration, reaching 6.5 and 10.1 μg/g in brain for the 2 respective doses. This was followed by a rapid decay in cocaine concentrations. Similar [31] or

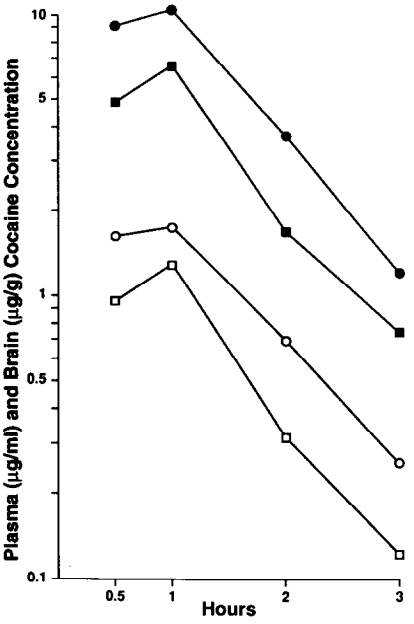

Fig. 3. Cocaine concentrations in plasma (open symbols) and brain (solid symbols) after subcutaneous administration of 20 (■, □) or 40 (●, ○) mg/kg doses in mice.

greater accumulation of cocaine in brain relative to plasma has been reported in dogs [31], mice [32–34], and rats [35,36]. However, other investigations using pregnant rats have found little [37] or no [38] accumulation of cocaine in brain. Indeed, the gestational state appears to significantly affect the pharmacokinetics of cocaine [32,39] and deserves further study.

The accumulation of cocaine in the brain may be primarily driven by a favorable partitioning of the drug from the circulation into the lipophilic environment of the brain. In addition, a receptor mediated component may also contribute to this accumulation as evidenced by the brain regional localization of the drug, *e.g.* within the striatum [40,41], a dopamine rich region which appears prominently involved in the stimulant effects of cocaine [42].

ACKNOWLEDGEMENTS

The authors thank Marty McDowell for her help in preparing this manuscript. This study was supported by Grant HHS No. 1R01DA08034.

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