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Dose–Response Cocaine Pharmacokinetics and Metabolite Profile Following Intravenous Administration and Arterial Sampling in Unanesthetized, Freely Moving Male Rats

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Abstract

Despite the wealth of experimental data on cocaine abuse, there are no published dose–response pharmacokinetic studies with bolus IV cocaine injection in the male rat. The present study examined the pharmacokinetics of arterial plasma concentrations of cocaine and metabolite profile [benzoylecgonine (BE), ecgonine methyl ester (EME), norcocaine (NC)] following a single IV injection of 0.5, 1.0, or 3.0 mg/kg cocaine. Male Sprague–Dawley rats ($N = 25$) were anesthetized and surgically instrumented with both jugular vein (drug administration) and carotid artery (blood withdrawal) catheters and allowed to recover for at least 24 h. Arterial plasma samples (200 μ l) were obtained at eight time points (0.5, 1, 1.5, 2, 5, 10, 20, 30 min) following IV bolus injection (15-s injection, 15-s flush) and analyzed by single ion monitoring using GC/MS. Nonlinear regression and noncompartmental pharmacokinetic analysis were employed. Mean $\pm$ SEM peak plasma concentrations of cocaine occurred at 30 s in a dose–response manner (370 ± 14 , 755 ± 119 , 2553 ± 898 ng/ml for 0.5, 1.0, and 3.0 mg/kg groups, respectively). $T_{1/2\alpha}$ was < 1 min for all groups, but inversely related to dose. $T_{1/2\beta}$ was independent of dose (13.3 ± 1.6 , 13.0 ± 1.5 , and 12.0 ± 2.0 min for 0.5, 1.0, and 3.0 mg/kg groups, respectively). MRT (16.0, 15.9, 14.5 min), V_{dSS} (3.3, 3.2, and 2.8 l/kg), and Cl_{TOT} (204, 201, and 195 ml/min/kg) also provided little evidence of dose-dependent effects. Although the metabolic profile of IV cocaine was similarly ordered for all dose groups (BE > EME > NC), a quantitative shift in metabolite profile was evident as a function of increasing dose. This metabolic shift, perhaps attributable to saturation of plasma and liver

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esterases, suggests that the recently reported pharmacodynamic effects positively correlated with IV cocaine dose are unlikely attributable to NC, a minor but pharmacologically active metabolite. In sum, the IV pharmacokinetic profile in rats is distinct from that observed via the SC, IP, and PO routes of administration and offers the potential to provide a reasonable clinically relevant rodent model.

Keywords

Cocaine; Drug administration routes; Pharmacokinetics; Rats, Sprague–Dawley; Benzoylecgonine; Ecgonine methyl ester; Norcocaine

A PRIMARY goal for the development of an animal model of drug abuse is to select a route of administration that closely mimics the pharmacokinetics of the drug observed in humans. For stimulant drugs commonly abused by humans via inhalation or intravenous injection, the often employed SC (9,64), IP (35,55), or PO (12,13) routes of drug administration in rats consistently fail to mimic the rapidly peaking pharmacokinetic profile observed in humans (17). Although rodent inhalation models are available for many drugs of abuse (5,7,19), the widespread adoption of the inhalation route of administration is limited by the practical, technical, and physiological issues associated with such exposures (50,73). Despite the fact that models for IV drug administration in rodents have been employed in self-administration studies for a number of years, the general applicability of these models has been constrained by the use of chronic indwelling catheters that are exteriorized and tethered (56,69,70). Nevertheless, consideration of the IV route of administration is important because it removes the process of absorption and provides near instantaneous distribution through the vasculature.

The development of an appropriate IV animal model for cocaine abuse has also been constrained by the protocols characterizing the clinical pharmacokinetic data. The often cited human studies that have utilized a bolus injection via the IV route of administration have employed male subjects, venous sampling, and drug administration—initial sampling intervals of 4–10 min (18,29–31). Although several human studies have employed initial sampling intervals beginning immediately (8), 2 min (3), or 5 min (39) after cocaine administration, these studies utilized an infusion protocol (2–3 min), which would be anticipated to both decrease and delay the peak plasma cocaine concentration (21). It now appears that the reliance of all of these human studies on venous sampling may have grossly underestimated the extent of brain exposure to cocaine. Evans and colleagues (17) have demonstrated that arterial sampled plasma cocaine concentrations are over 10 times greater than simultaneously obtained venous samples for either IV (bolus injection) or inhalation routes of administration. Moreover, rapid initial sampling indicated that peak arterial concentrations occurred within 15–30 s of injection whereas peak venous concentrations were not evident until 3–5 min. As stimulants like cocaine are abused for their euphoric effects and arterial samples provide a better estimate than venous samples of the concentration of cocaine delivered to the brain (33), the most relevant human data to model would appear to be those of Evans and colleagues (17).

Animal models of behavioral sensitization as well as those of maternal substance abuse have been particularly plagued by the confound of absorption and distributional factors. Behavioral sensitization is the increased behavioral response of rodents to repeated, intermittent, administration of cocaine (35). Although a variety of neurobiological mechanisms have been invoked to explain this phenomenon (36), critical roles of pharmacokinetic factors and dosing regimen have recently been identified. A 43% increase in brain cocaine levels was found to occur following 8 days of repeated IP (10 mg/kg) injections (53). In contrast, changes in brain cocaine levels did not occur when cocaine was administered to rodents IV (53,54). The increased brain levels following IP dosing appear to result from a difference in the absorption process from the site of the IP injection into the general circulation (54), thereby resulting in increased brain cocaine levels with repeated IP dosing. The use of the IV route of administration would eliminate the confound of altered absorption of cocaine and provide near instantaneous distribution throughout the circulation, thereby precluding any potential contribution of these pharmacokinetic factors to behavioral sensitization (53,68).

The study of maternal cocaine abuse via an IV rodent model would similarly benefit from removing absorption and distributional factors (46). The existing models with pregnant rats are characterized by sustained absorption with plasma levels greater than 1000 ng/ml for several hours (SC) (13,64), extraplacental absorption (IP) (11,16), and delayed absorption (15-min peak levels) with sustained plasma levels through 1 h (PO) (12,13). Furthermore, given that pharmacokinetic data in the pregnant woman are not obtainable, cross-species comparisons between humans and rats might be facilitated by a dose–response pharmacokinetic analysis of IV cocaine in the male rat. The only study of plasma levels of cocaine and BE after IV administration in the rat that directly quantified cocaine employed an infusion rather than a bolus injection protocol and used decapitation to obtain plasma samples (57). Although driven in part by the goal of obtaining “simultaneous” fetal samples, trunk blood is obviously a mixture of arterial and venous blood. To the best of our knowledge, there are no published data in the rat that have provided direct quantitation of cocaine, dose–response information, arterial sampling and injection sampling intervals of < 1 min. Given the recent availability of data in human male volunteers, which fulfills all of these criteria (17), we sought to provide a parallel data set in the male rat.

Accordingly, the goals of the present study were twofold. First, to determine the pharmacokinetic profile of arterial plasma concentrations of cocaine in the rat following a single IV bolus injection of 0.5, 1.0, or 3.0 mg/kg cocaine. Second, to assess the metabolic profile of cocaine by direct quantitation of BE, EME, and NC following the IV bolus injection of cocaine.

METHOD

Animals

Adult (11–12 weeks old; $N = 25$) male Sprague–Dawley rats (Harlan Sprague–Dawley, Indianapolis, IN) were group-housed and had access to both standard rat chow (Pro-Lab Rat, Mouse, Hamster Chow No. 3000) and water ad lib for a period of 2 weeks prior to the start of the experiment. The animals were placed in quarantine for 1 week and were then moved

to the animal colony. The animals were maintained according to NIH guidelines in AAALAC-accredited facilities. Specifically, the animal facility was maintained at $21 \pm 2^{\circ}\text{C}$, $50 \pm 10\%$ relative humidity, and had a 12-h light:12-h dark cycle with lights on at 0700 h (EST). The protocol for the use of rats in this research was approved by the IACUC of the University of Kentucky.

Chemicals

Cocaine HCl was purchased from Sigma Chemical Co. (St. Louis, MO) and was dissolved in sterile isotonic saline at the indicated concentrations based on the weight of the salt. All drugs solutions were prepared immediately prior to use in a volume of 1 ml/kg.

Experimental Design

The use of a conscious, chronically catheterized model was chosen over an anesthetized preparation as many common laboratory anesthetic agents may influence pharmacokinetic parameters as well as cytochrome P450 activity (23,24,41,44). All animals were surgically implanted with vascular catheters (as described below). Following at least 24-h recovery from surgery (20,23,24,26) the animals were randomly assigned to three groups ($n_s = 8-9$), which received a single IV injection of either 0.5, 1.0, or 3.0 mg/kg of cocaine. Arterial withdrawals commenced immediately after injection and were withdrawn periodically thereafter (0.5, 1.0, 1.5, 2.0, 5.0, 10, 20, and 30 min after the initiation of cocaine injection). The sampling intervals were chosen on the basis of 1) preliminary data (45), 2) previous reports (2,42,52,54,57), and 3) the expected half-life, as scaled from human data in the context of physiological time and metabolic rate (6,43,51). All animals were euthanatized after termination of the experiment.

Surgery

Catheterization was performed as previously described (46). Briefly, animals were anesthetized with a mixture of ketamine hydrochloride (100 mg/kg/ml) and xylazine (3.3 mg/kg/ml). The use of the ketamine/xylazine mixture was chosen over pentobarbital given the potent effect of the latter agent on cytochrome P450 activity (41). The jugular vein was exposed and a catheter (PE 50 Intramedic polyethylene tubing, Clay Adams, Parsippany, NJ) was tunneled under the skin from the back of the neck and inserted approximately 3 cm toward the right atria. The catheter was anchored with two sutures, one on either side of a silicone bead. A similar procedure was used to catheterize the carotid artery. Both catheters were sutured under the skin on the back, exteriorized (~4 cm), and sealed. Animals were kept under periodic postoperative observation and returned to the vivarium upon recovery from anesthesia. Animals were allowed to recover from surgery for a minimum of 24 h prior to the initiation of the experiment (20,23,24,26). Body weights were recorded prior to surgery and subsequently every day until the end of the experiment. The catheters were flushed twice daily with heparinized saline and the animals were observed for any signs of discomfort or behavioral distress.

Drug Treatment

Subsequent to implantation of the vascular catheters, the 25 implanted animals were assigned to one of three groups, which received either 0.5, 1.0, or 3.0 mg/kg of IV-administered cocaine. The cocaine was administered as a bolus injection delivered in a volume of 1.0 ml/kg (15 s) and was followed by flushing (15 s) with 0.2 ml heparinized (2.5%) saline (i.e., the approximate volume of the catheter). All arterial blood withdrawal sampling commenced immediately after injection.

Plasma Sample Collection

Blood samples (400 μ l) were removed via the indwelling arterial catheter at the eight specified time points (0.5, 1.0, 1.5, 2.0, 5.0, 10, 20, and 30 min) following the initiation of cocaine injection. All samples were collected into ice-chilled 1.5-ml microfuge tubes containing saturated NaF to prevent the hydrolysis of cocaine. Plasma samples (200 μ l) were obtained by centrifugation ($2040 \times g$ for 5 min) and then frozen at -80°C until assayed.

GC/MS Methodology

Chemicals and reagents—Cocaine, BE, EME, NC, and deuterated cocaine and BE were provided by Research Triangle Institute (Research Triangle Park, NC) courtesy of NIDA. Deuterated EME was from Radian Corporation (Austin, TX). Commercial standard solutions of cocaine metabolites at 1.0 mg/ml in methanol were from Alltech (State College, PA). Reagents were from Fisher (Pittsburgh, PA) and Pierce Chemical (Rockford, IL). Bond Elut columns were from Varian's Analytichem International Division (Harbor City, CA).

Sample extraction and chromatography—Samples were prepared according to a modification of procedures supplied by Analytichem. In brief, 100 μ l of freshly thawed homogeneous room temperature plasma was mixed with 50 μ l acetonitrile containing the internal standards 20 ng/ml benzoylecgonine- d_5 , 20 ng/ml cocaine- d_5 , 6.7 ng/ml ecgonine methyl ester- d_3 , and 2 ml 100 mM potassium phosphate buffer, pH 6.0. The vortexed mixture was applied to a methanol- and buffer-rinsed Bond Elut column and eluted by means of an SPS24 VacElut vacuum chamber at 1–2 ml/min. The column was rinsed with dH_2O , 100 mM HCl, and methanol, and the cocaine metabolites eluted with 2 ml freshly prepared CH_2Cl_2 /isopropanol/ NH_4OH (78:20:2) into a dry silanized tube. Dry dimethylformamide (20 μ l) was added to the extract as “keeper solvent,” the mixture transferred to a 1-ml Reactivial, and the extract evaporated to dryness at 40°C in a Pierce heating block under a stream of dry nitrogen gas. For derivatization of BE to its trimethylsilyl (TMS) ester, NC to its TMS-amine, and EME to its TMS ether, 50 μ l BSTFA + 1% TMCS was added to the vial, the capped vial was vortexed 30 s and then inserted into a 75°C heating block for 30 min. The cooled derivative was transferred to a 100- μ l glass insert-containing sample injector vial for automatic injection into a Hewlett-Packard 5890/5970 GC/MS with a 7673 autoinjector. The GC was equipped with a 30-m H-P DB5-MS column (0.25 mm i.d., 0.25 μ m film thickness) with a flow rate of less than 1 ml/min helium. The derivatized extract (1 μ l) was injected into a 250°C injector port equipped with a silanized quartz insert, and the GC column was programmed from 150 to 290°C at $20^{\circ}\text{C}/\text{min}$ after an initial 1 min at 150°C . The transfer line was kept at 280°C . Metabolite

retention times under this regimen were typically 4.3 min (EME-TMS), 7.9 min (cocaine), 8.2 min (BE-TMS), and 8.4 min (NC-TMS).

Mass spectrometric analysis—Cocaine and metabolites were prepared from dry powders as concentrates in acetonitrile for generation of a standard curve including concentrations 0, 20, 50, 100, 200, 500, 1000, 2500, 5000, 7500, and 10000 ng/ml. The accuracy of the resultant curve was validated using 70, 400, and 2000 ng/ml standards obtained by dilution of 1.0 mg/ml solutions from Alltech. Dynamic mass calibration identified the following stable peaks as appropriate to each compound undergoing mass spectral analysis on our instrumentation: EME-TMS: 271.1, 256.1, 240.1, and 182.0; EME- d_3 -TMS: 274.1; cocaine: 303.1, 272.0, 182.0, and 198.0; cocaine- d_5 : 308.1; BE-TMS and NC-TMS: 361.1, 346.1, 256.1, and 240.1; BE- d_5 -TMS: 366.1. Metabolites were identified by the occurrence of their chromatographic peaks within windows having no greater than 5% deviation from the retention time of standards, by their coelution with deuterated peaks in the cases of EME-TMS, cocaine, and BE-TMS, and by no greater than 10% deviation from anticipated area ratios characteristic of each compound (EME-TMS: 271.1/256.1, 271.1/240.1, 271.1/182.0; cocaine: 303.1/272.0, 303.1/182.0, 303.1/198.0; BE-TMS and NC-TMS: 361.1/346.1, 361.1/256.1, 361.1/240.1). Ratios of molecular ion abundance to that of the deuterated standard's molecular ion provided the quantitation relevant to generation of the standard curve: EME: 271.1/274.1; cocaine: 303.1/308.1; BE: 361.1/366.1; and NC: 361.1/308.1 (cocaine- d_5). Each calibration point was prepared in triplicate and the average plotted against known concentration for generation of the standard curve. Linear regression analysis enabled interpolation of unknown values. Assay sensitivity was 10 ng/ml for cocaine and each of the metabolites. Assay coefficients of variation were 6%, 3%, 6%, and 17% for cocaine, BE, EME, and NC, respectively.

Pharmacokinetic Calculations

Curve fitting to a two-compartment open model was performed by derivative-free nonlinear regression with replicates ("repeated runs") (14) for each set of animals that received the same IV cocaine dose (AR, BMDP Statistical Software, Release 7, Los Angeles, CA). This nonlinear regression analysis provided the parameters of $T_{1/2\alpha}$ and $T_{1/2\beta}$. Pharmacokinetic model parameters of AUC (0– ∞) and AUMC (0– ∞) were subsequently determined with the PKAnalyst program (MS Windows version of RSTRIP II, Micromath Inc, Salt Lake City, UT). Standard noncompartmental pharmacokinetic formulas were used to compute MRT (min), V_{SS} (l/kg), and Cl_{TOT} (ml/min/kg) (21).

An individual concentration measurement was considered an outlier if it was 4 times > or < than the mean of all concentrations taken at the same time within the same dose group. The outliers (4, 0, and 2 for the 0.5, 1, and 3 mg/kg dose groups, respectively) were replaced by values predicted for the time point by interpolation of the concentrations measured at the immediately preceding and following time points.

Data Analysis

Body weight data were analyzed by one-way ANOVA. Arterial plasma cocaine concentrations were analyzed by a 3 dose X 8 sampling interval ANOVA with sampling

interval as a within-subject factor (4V, BMDP Statistical Software, Release 7, Los Angeles, CA). All GC/MS metabolite AUC (T_0 to T_{30} min) data were similarly subjected to a 3 dose $\times$ 3 metabolite ANOVA with metabolite as a within-subject factor. The Greenhouse-Geisser *df* correction factor was used for violations of compound symmetry (22). Orthogonal decomposition and specific linear contrasts were also employed to evaluate the potential dose-dependent effects of the IV cocaine treatment. An α level of $p < 0.05$ was the significance level set for rejection of the null hypothesis.

RESULTS

Complete sets of serial samples were obtained from 22 of the 25 catheterized rats. Average body weight of the animals was 324.6 ± 12.8 g and there was no significant difference among the average weights of the cocaine dose groups, [$F(2, 19) < 1.0$].

The IV administration of cocaine with carotid artery sampling initiated immediately after termination of drug injection produced a pronounced but transient dose-response peak in arterial concentrations with apparent dose-independent elimination kinetics (Fig. 1). An ANOVA of the cocaine concentration data from T_0 to T_{30} indicated significant effects of dose, [$F(2, 19) = 17.9, p = 0.001$], sampling interval, [$F(7, 133) = 14.8, p = 0.001$], and an interaction of dose and sampling interval, [$F(14, 133) = 4.3, p = 0.03$], that was characterized by prominent linear trends [linear dose $\times$ sampling interval effect, [$F(1, 19) = 9.0, p = 0.007$]. The mean $\pm$ SEM peak plasma concentrations of cocaine occurred at 30 s and were proportional to the administered IV dose [$370 \pm 14, 755 \pm 119, 2553 \pm 898$ ng/ml for 0.5, 1.0, and 3.0 mg/kg groups, respectively; $r = 0.60, F(1, 20) = 11.1, p = 0.004$]. By the 20-min postinjection sample interval the arterial plasma concentrations of all dose groups had decreased by an order of magnitude ($34 \pm 5, 72 \pm 5, 217 \pm 13$ ng/ml for 0.5, 1.0, and 3.0 mg/kg groups, respectively).

The pharmacokinetic parameters for the bolus IV injection of cocaine at doses of 0.5, 1.0, and 3.0 mg/kg, as determined by derivative-free nonlinear regression and noncompartmental analysis, are given in Table 1. Comparable to the peak arterial cocaine concentration data, the AUC (T_0 – ∞) and AUMC (T_0 – ∞) measures were also clearly proportional to the administered IV dose. The trapezoidal AUC (T_0 – T_{30}) represented 86–88% of the AUC (T_0 – ∞) for each of the dose groups and thus suggested that extrapolation to infinity did not compromise the between-group dose comparisons (40). Cocaine distribution was extremely rapid and occurred with a half-life of less than 1 min for all dose groups; however, there was a significant dose-dependent decrease in $T_{1/2\alpha}$ with increasing IV dose ($p = 0.01$). In contrast, the estimates of $T_{1/2\beta}$ were independent of dose ($13.3 \pm 1.6, 13.0 \pm 1.5, \text{ and } 12.0 \pm 2.0$ min for 0.5, 1.0, and 3.0 mg/kg groups, respectively). The noncompartmental parameters of MRT (16.0, 15.9, 14.5 min), V_{dSS} (3.3, 3.2, and 2.8 l/kg), and Cl_{TOT} (204, 201, 195 ml/min) also provided no convincing evidence of dose-dependent effects.

The metabolic profile for the 3.0 mg/kg dose group is illustrated as a set of concentration vs. time functions in Fig. 2. The relative ordering of the metabolites indicated that BE was the primary metabolite, with EME values approximately 1/10 that of BE and NC values 1/40 that of BE. Figure 3 provides a semi-log plot of the AUC for the concentration vs. time

function from T_0 to T_{30} min for all of the metabolites. The metabolite profile displayed the same ordinal relationship across the 0.5–3.0 mg/kg dose range (BE > EME > NC); however, there was a quantitative shift of metabolite concentrations as a function of increasing dose. The relative metabolite profile for BE, EME, and NC was, respectively, 52%, 26%, and 22% for the 0.5 mg/kg dose, 61%, 21%, and 18% for the 1.0 mg/kg dose, and 89%, 8%, and 3% for the 3 mg/kg dose. Statistical comparisons among the AUC indices of the metabolite profiles confirmed a significant interaction of IV cocaine dose and metabolite concentration, [$F(4,38) = 5.1, p = 0.005$, and further indicated this interaction was characterized by prominent linear trends [linear metabolite $\times$ linear dose effect, [$F(1,19) = 9.0, p = 0.007$].

DISCUSSION

The most common routes of cocaine abuse by humans are inhalation, injection, and insufflation, with similar pharmacokinetic and pharmacodynamic effects characterizing the two former routes (17,18). Dose-response pharmacokinetic studies of cocaine in the rat have not previously been published for the IV route of administration, although single-dose studies (2,42,52,54,57,67) and an inhalation study (5) with IV reference data ($ns = 2-3$) are available. The administration of cocaine as an IV bolus injection to unanesthetized, freely moving rats produces a pharmacokinetic profile distinguished by a rapid transient peak drug concentration associated with a $T_{1/2\alpha}$ of < 1 min, proportional increases in plasma drug concentration as a function of dose, dose-independent elimination kinetics, and a metabolite profile of BE > EME > NC.

The IV concentration vs. time profile is markedly distinct from that observed in rats when cocaine is administered via the SC (13,64), IP (11,16), or PO (12,13) routes. Not only were the peak levels comparable or lower than those of the alternative routes (~2500 vs. ~2300, ~2200, or ~5400 ng/ml, respectively) and obtained more rapidly (30 s vs. 120, 30, or 15 min, respectively), but they were also followed by a precipitous clearance. The average duration of cocaine levels above 1000 ng/ml for the IV route was 60–90 s vs. 120, 90, or 60 min for SC, IP, and PO routes, respectively. A transient, rapidly peaking concentration profile has also been reported in pregnant rats following a 6 mg/kg IV dose of cocaine administered over a 90-s infusion (57) as well as in male rats following either a 6 or 10 mg/kg IV dose of cocaine of unspecified duration (5).

The recent report in adult male humans of a rapid transient peak in plasma cocaine levels within 15–30 s of injection with arterial sampling (~2500 ng/ml) stands in marked contrast to the magnitude and time course of peak plasma concentration of venous sampling (17). The simultaneously obtained venous samples indicated maximal values at 3–5 min after the 15-s bolus IV injection (~250 ng/ml)—values quite consonant for that dosage (~0.5 mg/kg) with the often-cited human studies (3,29,30) that provided the basis for much clinical and basic cocaine research spanning nearly two decades. The concentration vs. time profile in other mammalian species, typically obtained via arterial sampling, also displays a transient peak [guinea pig (59); dog (72); sheep (10,38,49,74); pig (37,61); monkey (58)]. The importance of considering arterial, as opposed to venous, sampling for the study of stimulant drugs of abuse such as cocaine derives from the fact that arterial concentrations are those that are delivered to the brain (33)—the putative basis for why these drugs are abused.

The estimation of $T_{1/2\alpha}$ with IV injection has been relatively infrequently reported. The typical injection-sampling interval of 4–5 min in human studies in conjunction with monoexponential modeling has precluded the determination of a separate distribution phase. Notable exceptions that employed biexponential models indicated a $T_{1/2\alpha}$ of 20–40 min (39) or 13 min (3), although the initial venous blood sample was delayed for 5 min after injection. It remains possible that a very rapid distribution phase of cocaine was missed in these early studies because blood samples were not taken soon enough after IV administration (8). A particularly noteworthy exception, based on a pharmacokinetic analysis of mean arterial sampled concentration data (17), suggests a $T_{1/2\alpha}$ estimate for male human volunteers of < 1 min for both 16 and 32 mg doses. Similarly, in the present study, estimates of $T_{1/2\alpha}$ were < 1 min for all dose groups with sampling begun immediately after termination of injection. When initial blood samples are drawn, arterially, prior to 1 min after injection, estimates of $T_{1/2\alpha}$ approximate 1 min or less [pigs (61); rat (5,67), calculated; sheep (10), calculated]. With initial blood samples drawn between 1 and 5 min after bolus injection, estimates of $T_{1/2\alpha}$ approximate 1–3 min [guinea pig (59), calculated; dog (72); rat (42)]. Despite the obvious dependence of the estimates of $T_{1/2\alpha}$ on experimental design (e.g., timing and frequency of sampling), the general markedly rapid distribution is perhaps attributable to the physiochemical properties of cocaine (62). Although interpretation of the inverse relation of $T_{1/2\alpha}$ to dose in the present study would benefit from more frequent early samples, the 30-s sampling interval was as rapid as we could reliably withdraw the initial four blood samples.

Estimates of the $T_{1/2\beta}$ of cocaine are among the most commonly reported kinetic parameter. The values for the male rat in the present study were 12–13 min for doses of 0.5–3 mg/kg. Prior reports with IV administration in male rats have suggested $T_{1/2\beta}$ estimates of 15 ± 2 min ($n = 6$, 1 mg/kg dose) (2), 19 ± 3 min ($n = 9$, 2 mg/kg dose) (42), 14 ± 1 min ($n = 5$, 7.5 mg/kg dose) (54), and 18 min ($n = 3$, [^3H]-8 mg/kg dose) (52). A $T_{1/2\beta}$ value of approximately 22 min was calculated from the mean data obtained in pregnant rats given a 90-s infusion of cocaine ($n = 5$ –12, 6 mg/kg dose) (57), whereas a $T_{1/2\beta}$ value of approximately 10 min was derived from the mean data obtained in male rats given a bolus injection (unspecified duration) of cocaine ($n = 6$ –10, 3 mg/kg dose) (67). Prolonged $T_{1/2\beta}$ estimates of 79–88 min were obtained from a triexponential curve fit (5); $T_{1/2\beta}$ estimates based on the second exponential term were either 27 min ($n = 2$, 6 mg/kg) or 8 min ($n = 3$, 10 mg/kg). Given the likely possibility of dose-dependent kinetics at convulsive doses (38,59) (5 mg/kg is uniformly convulsant in 8/8 rats within 30–60 s, whereas 10 mg/kg approaches the LD_{50} , Mactutus, unpublished data) the physiologically relevant half-life estimates (4) in rats appear to be in the range of 10–19 min.

$T_{1/2\beta}$ estimates of IV cocaine in humans range from approximately 40–168 min with a consensus of 40–50 min (3,8,18,29–31,39); the extreme 168 min estimate (39) may be considered inaccurate given the inability of the assay employed to reliably measure low cocaine plasma concentrations (3). In light of the three to fourfold difference in circulation time of the rat relative to a 70 kg human (43), the 12–13 $T_{1/2}$ estimate of the present data is in reasonably good agreement with the human data.

The noncompartmental pharmacokinetic parameters of Cl_{TOT} , Vd_{SS} and MRT suggested, similar to the estimates of $T_{1/2\beta}$, dose-independent kinetics across the dose range of 0.5 to 3 mg/kg. A Vd_{SS} of 2–4 l/kg suggests that there is moderate distribution of cocaine to bodily tissues. The Vd_{SS} estimates of the present study (2.8–3.3 l/kg) are similar to previous reports for doses of 1 mg/kg (3.61 l/kg) (2) and 2 mg/kg (2.7 l/kg) (42) of cocaine administered IV to the rat; however, they are markedly less than the 16–17 l/kg values obtained with IV dosing of 6 or 10 mg/kg (5). The Cl_{TOT} rate of 200 ml/min/kg, although 7–10-fold greater than the rate for humans on an absolute scale, is consistent with the principle that smaller species deliver drugs faster and more frequently to the eliminating organs (43). Cardiac output in the unanesthetized adult male rat (333 g) averages 29.2 ± 4 ml/min/100 g (63), and thus substantially exceeds the present estimates of Cl_{TOT} (20 ml/min/100 g). Previous reports of Cl_{TOT} rates in the rat for IV cocaine doses of 1 and 2 mg/kg were 142 ml/min/kg (2) and 123 ml/min/kg (42), respectively. With IV dosing of 6 or 10 mg/kg, Cl_{TOT} values of 233–265 ml/min/kg were obtained (5).

The second aim of the study was to assess the metabolic profile of cocaine by direct quantitation of BE, EME, and NC following the IV bolus injection of cocaine. BE and EME are the principal metabolites of cocaine in humans (27,28). BE results from the nonenzymatic hydrolysis of cocaine whereas EME is formed by the action of plasma and liver esterases on cocaine. The formation of liver-derived metabolites, principally the demethylated product NC, is estimated to be 2–6% of cocaine in humans (27). Ion cluster analysis in conjunction with GC/MS has previously shown that the metabolite profile of cocaine appears quite similar in humans and rats (32). The present data in the rat indicate that BE and EME represent between 52% and 89% and 8% and 26% of the total metabolite profile, respectively. Neither BE or EME was shown to have pharmacological activity following IV doses in rats as high as 200 mg/kg (47). Substantial NC levels are of potential significance because this metabolite appears equipotent with cocaine in inhibiting [3H]NE brain synaptosomal uptake (25) and may have greater local anesthetic potency than cocaine (34). Plasma NC concentrations of 3% that of cocaine were reported following a 30 mg/kg IP dose delivered to pregnant rats (11). In the present study, with the 3 mg/kg dose of cocaine that mimics the transient arterial peak of ~2500 ng/ml observed in humans with 32 mg of cocaine (17), NC represented approximately 3% of the total metabolite profile.

The metabolite profile displayed the same ordinal relationship across the 0.5–3.0 mg/kg dose range (BE > EME > NC); however, there was a quantitative shift in metabolite concentrations with increasing cocaine dose. At the 0.5 mg/kg dose, the relative metabolite profile was 52%, 26%, and 22% whereas at the 3 mg/kg dose the profile shifted to 89%, 8%, and 3%, for BE, EME and NC, respectively. One possible explanation for this metabolic shift ($\uparrow$ BE, $\downarrow$ EME, $\downarrow$ NC) is the saturation of plasma and liver esterases, although the failure to note any dose-dependent changes in clearance might argue to the contrary. Nevertheless, it is generally accepted that the enzymatic hydrolysis of cocaine to EME is most prominent when plasma concentrations are a few hundred ng/ml, whereas appreciable nonenzymatic hydrolysis to BE occurs in vivo with cocaine concentrations greater than 1000 ng/ml (62). Although the decrease in NC with increasing dose appears contrary to that noted by monitoring $^{14}CO_2$ exhalation after administration of *N*-[^{14}C]methyl-labeled cocaine (65),

the $V_{\max}$ for NC hydrolysis is greater than that for cocaine by a factor of 1.7 times (serum) and 3.2 times (liver) (66). Another contributing determinant may be that a soluble metabolite of cocaine inhibits cocaine *N*-demethylation (60). Although the basis of this metabolic shift was not addressed, it nevertheless suggests that any pharmacodynamic effects positively correlated with IV dose of cocaine [e.g., the linear dose-dependent effects of IV cocaine to increase total locomotor activity (68)] are unlikely to be attributable to confounding due to elevated plasma levels of NC.

Application of the strategy commonly employed for therapeutic agents (i.e., reproducing the average plasma levels for a sustained period of time) has lead to animal models that most closely mimic those of a chronic cocaine user with a daily habit. Autopsy studies of recreational cocaine users dying of drug overdose have revealed an average plasma cocaine concentration of 6000 ng/ml (71). Similar postmortem blood cocaine levels of up to 4000, 4500 and 5600 ng/ml have also been reported after recent cocaine use (1). However, if one is to mimic at or below levels of occasional recreational use, the administration of cocaine should reproduce transient peak arterial levels of approximately 2500 ng/ml within 30 s with a distribution half-life of < 1 min (17). Collectively, the present data suggest that bolus IV cocaine administration in rats provides a viable alternative to SC, IP, or PO routes for providing a clinically relevant rodent model. However, potential changes in the pharmacokinetics of cocaine as a function of chronic IV injection (54,57) or pregnancy (15,48,75) await to be addressed.

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FIG. 1.

The concentration (mean $\pm$ SEM) vs. time curves were proportional to dose for IV cocaine administered as a bolus injection to unanesthetized, freely moving adult male rats. Arterial samples were drawn, beginning immediately after the 30-sec IV cocaine bolus (including catheter flush) and periodically throughout 30 min.

FIG. 2.

Concentration (mean $\pm$ SEM) vs. time curves for cocaine and major (BE) as well as minor metabolites (EME and NC) are shown for the animals that received an IV bolus injection of 3 mg/kg cocaine. The relative ordering of the metabolites indicated that BE was the primary metabolite, with average EME values approximately 1/10 that of BE and average NC values approximately 1/40 that of BE.

FIG. 3.

A dose-dependent shift in metabolite profile is illustrated as indexed by the area under the concentration (mean $\pm$ SEM) vs. time curve from T_0 to T_{30} . Although the AUC for the concentration vs. time function from T_0 to T_{30} for each of the metabolites was similarly ordered for all dose groups (BE > EME > NC), a significant shift in metabolite concentrations was evident as a function of increasing cocaine dose. Whereas BE was increased, both EME and NC were depressed at the 3.0 mg/kg dose.

TABLE 1

PHARMACOKINETIC ANALYSIS SUMMARY FOR IV COCAINE

Parameter	IV Dose of Cocaine		
	0.5 mg/kg	1.0 mg/kg	3.0 mg/kg
<i>n</i>	7	8	7
Body weight (g)	309 ± 24	325 ± 20	340 ± 25
Pseudo R^2	0.99	0.98	0.96
$T_{1/2\alpha}$ (min) *	0.9 ± 0.1	0.7 ± 0.2	0.4 ± 0.1
$T_{1/2\beta}$ (min)	13.3 ± 1.6	13.0 ± 1.5	12.0 ± 2.0
AUC (T_0 - ∞)	2446	4983	15348
AUMC (T_0 - ∞)	39212	79091	222197
MRT (min)	16.1	15.9	14.5
Cl_{TOT} (ml/min/kg)	204.4	200.7	195.5
Vd_{SS} (l/kg)	3.3	3.2	2.8

All data are means; estimates of variability are standard deviations.

The lack of fit (ANOVA) based on replicates failed to achieve statistical significance for each of the dose groups: 0.5, $F(4, 48) = 2.4$; 1.0, $F(4, 56) = 1.0$; and 3.0, $F(4, 48) = 0.5$.

* All groups significantly different from each other by Bonferroni *t*-test ($p < 0.01$).