Oral Cocaine Pharmacokinetics and Pharmacodynamics in a Cumulative-Dose Regimen: Pharmacokinetic-Pharmacodynamic Modeling of Concurrent Operant and Spontaneous Behavior within an Operant Context

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ABSTRACT

Despite wide use of cumulative-dosing procedures to evaluate dose-response relations, limited attention has been paid to investigating drug concentration-effect relations. We first characterized the pharmacokinetic (PK) parameters for i.v. (2 mg/kg) and oral cocaine (20 and 40 mg/kg) in rats. Cocaine's concentration-time profile for the escalating cumulative-dose regimen was simulated from PK parameters, dose size (1, 2, 7, 20, and 45 mg/kg by the oral route), and dosing interval (t, 35 min) as well as validated from blood sampling at various time points. This concentration-time profile was integrated with pharmacodynamic (PD) profiles of differential reinforcement of low rate performance and spontaneous activity (large and small movements) under a differential reinforcement of low rate 45-s schedule. Effects on three behavioral measures were characterized by integrated PK-PD models using the sigmoid $E_{\text{max}}$ (for increases in shorter response rate or large movements) and inhibitory $E_{\text{max}}$ (for decreases in density of reinforcement) models. But for the intrinsic differences in baseline and efficacy values among the behavioral endpoints, one set of PD parameters (i.e., potency and Hill factors) predicted concentration-effect relations for the three behavioral indices across all five doses. Concurrent monitoring of operant and spontaneous activity behavior within an operant context provides a novel behavioral paradigm to investigate drug effects on spontaneous activity under conditions where a behavioral contingency exists. Additionally, a cumulative-dosing procedure is efficient for determining the entire dose-response relation and provides an ideal mode to study phenomena such as sensitization or tolerance by varying dose size and/or $t$. The effects of drugs on behavior are commonly expressed by dose-response relations. However, constructing dose-response curves may be a lengthy procedure if each session measures effects from only one dose, and if drug sessions must be spaced several days apart to minimize “carryover” effects between administrations. Alternatively, drug dose-response relations may be determined by cumulative-dosing procedures consisting of multiple subsessions separated by time-out periods (e.g., 3 min) during which all experimental contingencies are turned off and doses administered. Thus, cumulative dosing can save substantial time in determining dose-response curves. Because the introduction of cumulative dosing in whole-animal preparations (Boren, 1966; Hanson et al., 1966), this procedure has been frequently used to evaluate drug effects (Wenger, 1980; Thompson et al., 1983; Spealman et al., 1989; Walker and Branch, 1998); however, in behavioral pharmacology, limited attention has been paid to investigating drug concentration-effect relations. Unlike dose-effect relations plotting collapsed time course effects as single points against administered dose, concentration-effect relations integrate effect-time profiles with serum concentration-time profiles (CTPs) and plot effects against bioavailable dose in the form of concentration (Sun and Lau, 2000), facilitating the comparison of potency across drugs or routes of administration and the detection of involvement of active metabolites in drug action for the parent compound.

ABBREVIATIONS: CTP, concentration-time profile; DRL, differential reinforcement of low rate; PD, pharmacodynamics; PK, pharmacokinetics; IRT, inter-response time; t, dosing interval; RM, repeated measures; AIC, Akaike’s information criterion; $V_c$, volume of distribution in the central compartment; Cl, clearance; $V_{ss}$, volume of distribution at steady state; $EC_{50}$, the concentration at half of $E_{\text{max}}$ for the shorter-response rate or large movements; $E_{\text{max}}$, the maximal effect; $IC_{50}$, the concentration at half of $E_{\text{max}}$ for the density of reinforcement; lm, large movements; CV, coefficient of variation.
Both the dose-response and concentration-effect relations of i.v., i.p., and p.o. cocaine were investigated under a differential reinforcement of low rate (DRL 45-s) schedule using a noncumulative-dosing procedure (Lau et al., 1999a; Ma et al., 1999a,b). In those studies, cocaine pharmacodynamics (PD) was closely related to its pharmacokinetics (PK); the shorter response rate [i.e., rate of responses with shorter inter-response times (IRTs) < 45 s] and density of reinforcement [i.e., IRTs ≥ 45 s; in reinforcements/min] were the two PD measures monitored. In another study (Lobaringas et al., 1999), the dose-response relation for oral cocaine was determined in one session consisting of five 35-min subsessions separated by 3-min time-outs during which doses of 1, 2, 7, 20, and 45 mg/kg were administered, producing added-up dose sizes of 1, 3, 10, 30, and 75 mg/kg. However, cocaine concentration-effect relations were not determined. To do so, a strategy was developed to trace detailed drug CTPs as concentrations increased and then decreased after each administration, although the extent of this fluctuation mainly depends upon PK parameters, dose size, and dosing interval (τ). To detect concentration fluctuations within multiple dosing, more frequent blood sampling is required after administration of five escalating doses.

This study aimed, therefore, to apply PK principles developed by pharmacokineticists in therapeutics to describe and predict the oral cocaine CTP in the above-mentioned cumulative-dose regimen. An escalating cumulative-dosing procedure, used commonly in behavioral pharmacology, is a special case of a multiple-dose regimen. Drug CTPs for multiple dosing with various dose sizes in the linear range and τ values can be predicted once a drug’s PK parameters have been characterized (Gibaldi and Perrier, 1982). We first characterized PK parameters for oral cocaine to simulate CTP for the cumulative-dose regimen, followed by CTP validation. Once CTP was defined mathematically, concentration-effect relations of the two PD measures under the DRL 45-s schedule were determined by PK-PD modeling. As executed, the proposed PK model facilitates the design of other dose regimens for maintaining desired steady-state concentrations for partitioning PK determinants from observed effects. Furthermore, the complete CTP can explain the need for caution when comparing the effects of a specific dose within a cumulative-dose regimen with those from a single-dose regimen; this kind of comparison is often included in behavioral analyses (Wenger, 1980; Bertalmio et al., 1982; Terry, 1992; Schechter, 1997).

In addition to investigating cocaine’s effects on DRL performance, we concurrently examined effects on spontaneous activity by placing each operant chamber atop an activity platform. Spontaneous activity is generally considered unconditioned behavior that involves no behavioral contingency. Cocaine’s effects on spontaneous activity within the operant context, however, may reflect the drug’s effects on general activity levels in humans in which cocaine is administered in real-life contingency contexts. Concurrent monitoring of operant and spontaneous behavior within an operant context provides a novel behavioral paradigm to investigate drug effects on spontaneous activity under conditions wherein a behavioral contingency is in effect. An added advantage of the combinatory paradigm is that it enables one to investigate the interaction between operant and spontaneous activity behavior under the influence of cocaine. We found that the development of acute tolerance to cocaine’s psychomotor stimulant effect is largely dependent on whether a contingency is involved in the behavioral paradigm using PK-PD modeling (Lau et al., 1999b).

Materials and Methods

PK of Cocaine and Its Metabolites

Animals. Four male albino, Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN,) with a mean, initial body weight of 383 g (range: 380–386 g) were used. They were housed individually in a temperature-regulated room with a 12-h light/dark cycle (lights on at 7:00 AM). Animal body weight was reduced to 80% of free-feeding levels by limiting daily food rations over a 2-week period as described previously (Lau et al., 1999a), and held at this weight for 3 months before the start of the experiment, a time period required to train and establish baseline performance under the DRL 45-s schedule. Water was made continuously available in the living cages. All experiments were executed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication 85-23, revised 1985).

Drug. Cocaine HCl was obtained from the Research Triangle Institute (Research Triangle Park, NC) through the National Institute on Drug Abuse. The drug was dissolved in 0.9% NaCl and administered either i.v. or p.o. by gavage in a volume of 1 ml/kg body weight. When an i.v. bolus dose of cocaine was administered, the drug solution was delivered in 30 s and followed by injection of 0.3 ml of 0.9% saline in 30 s. For oral administration of cocaine, feeding needles with 4-mm ball-tipped stainless steel no. 14, 7.7 cm were used. All cocaine doses are expressed in terms of the salt and were corrected to cocaine base for the calculation of PK parameters.

Reagents and HPLC. Cocaine’s metabolites (norcocaine and benzoylecgonine) and cocaethylene fumarate were also obtained from National Institute on Drug Abuse. Reagents were obtained from standard commercial sources. The serum microsample UV HPLC method for determination of cocaine and its metabolites has been described previously (Ma et al., 1997), with the exception that a fluorescence detector (Hewlett Packard, Waldbronn, Germany) was used, replacing the UV detector. The fluorescence detector provides improved sensitivity and selectivity over the UV detector used previously for quantifying cocaine and its metabolites (Sun et al., 2000) because it selectively detects the weak native fluorescence of the benzene ring present in the molecules of cocaine and its metabolites. Accordingly, we selected an internal standard containing a benzene ring for this updated method. The excitation and emission wavelengths for the fluorescence detector were set at 230 and 315 nm, respectively. The capacity factors for benzoylecgonine, cocaine, norcocaine, and cocaethylene (as an internal standard) were 1.89, 3.60, 4.95, and 7.90, respectively. The within-day and between-day precisions of this method for all compounds at four concentrations (0.05–1.00 μg/ml) were high with the coefficients of variation (CV) within the range of 1.20 to 7.82% and 1.78 to 7.23%, respectively; the detection limit was 0.5 ng/ml.

Drug Administration and Blood Sampling. Animals were implanted with right jugular vein catheters as described previously (Ma et al., 1999a). Immediately after the jugular vein catheterization, right femoral vein catheters were also implanted. The dual catheters allow administration of i.v. cocaine via the femoral vein catheters with blood sampling from the jugular vein catheters to avoid contamination of the blood samples with dosing solution. The animals were allowed to recover from catheterization for at least 2 days before the cocaine dosing series.

The PK experiment was conducted in four Plexiglas chambers similar in size to those used for the behavioral experiment. Each chamber was equipped with a stainless steel food-pellet receptacle into which 45-mg dustless pellets could be delivered. Animals first received an i.v. bolus 2-mg/kg dose and, on different days, a p.o. bolus...
20- and a 40-mg/kg dose. Blood samples (100 μl) were obtained via jugular vein catheters at 5, 10, 20, 30, 60, 90, and 120 min post injection. Animals also received an escalating oral cocaine dosing series (i.e., 1, 2, 7, 20, and 45 mg/kg) with each dose separated by 35-min intervals. This escalating cocaine dosing series produced added-up or cumulative dose sizes of 1, 3, 10, 30, and 75 mg/kg. Blood samples were obtained at the 15-min time point immediately after administration of each of the 1-, 2-, and 7-kg/ mg/kg doses of cocaine; however, an additional blood sample was also obtained at the 35-min time point after the 20-mg/kg dose. After administration of the last cocaine dose (45 mg/kg), blood samples were obtained not only at 15 and 35 min but also at 60 min. An equal volume of sterile saline was administered to maintain a constant blood volume after collection of each blood sample. Collectively, animals received a total of four cocaine-dosing sessions (i.e., three bolus and one cumulative dosing) with each session separated by 3 to 5 days.

To minimize group differences between PK and PD studies, animals in PK group received 45-kg food pellets matched in mean number at the 15- and 35-min time points with those earned during the corresponding DRL 45-s sessions after each cocaine administration for the cumulative-dosing series. For the three bolus doses, the mean matched food pellets given at time points of 15, 30, 45, 60, 90, 120, and 180 min were derived from a previous study (Ma et al., 1999a). If feeding and blood sampling times overlapped during an experiment, food pellets were always given immediately after a blood sampling procedure. After the last blood samples were taken, animals were returned to their home cages and given food rations sufficient to maintain their usual, daily criterion weights.

Blood samples were centrifuged for 10 min at 13,700 g; serum samples were stored frozen (-50°C) until analysis. Previously, we have found that in vivo rat serum cocaine samples were stable for at least a month without the presence of sodium fluoride, a cholinesterase inhibitor (Lau et al., 1990). Thus, sodium fluoride was not used in the present study because serum samples were analyzed within a week.

**PD: DRL 45-s Performance and Spontaneous Activity**

**Animals.** Eight male rats with initial body weights of 379 g (range: 373–383 g) of the same strain were placed under conditions similar to those used in the PK study, including the food-limited regimen. Each of these animals had previously responded under the DRL 45-s schedule and had received oral d-amphetamine.

**Apparatus. DRL 45-s schedule.** Each of the four experimental chambers, equipped with a response lever and a stainless steel food-pellet receptacle into which 45-mg dustless pellets (BioServ, Frenchtown, NJ) could be delivered, was enclosed in a sound-attenuating shell and controlled by an IBM-type 586 X computer. Session contingencies were programmed and data recorded using QuickBasic.

**Spontaneous activity.** Each experimental chamber was secured atop an individual activity platform. The four platforms were outfitted with a precision linear load cell transducer with an adjustable gain amplifier, 60-Hz notch filter, and a fitted base (PHM-252A-60; Med Associates, Georgia, VT). The platforms were linked to a high-speed analog to digital converter (DIG-729 ADC; Med Associates), which was connected to a 586 IBM-compatible computer. The maximal and minimal crossing thresholds for each platform’s load cell amplifier were adjusted using a calibration unit (PHM-252c; Med Associates). The minimal threshold was used for monitoring grooming and head movements (i.e., small movements), whereas the maximal threshold was used for locomotor activity and rearing (i.e., large movements). Large and small counts were measured in terms of converted analog to digital crossings and time spent above the threshold crossings. Data was recorded using the Threshold activity program (SOF-805A; Med Associates). To avoid interference of lever presses with activity monitoring, the program was configured to delete the activity recordings that occurred during the 600 ms immediately preceding and following a lever press.

**Procedure. DRL 45-s schedule.** Rats were trained to respond under a DRL 45-s schedule as described previously (Lobarinas et al., 1999). Each 190-min session consisted of five 35-min subsessions with each subsession preceded by a 3-min time-out period during which treatments could be administered. During the time-out periods, the house lights were turned off and lever responses had no consequences. Animals were exposed to daily 190-min experimental sessions for the duration of the experiment.

**Spontaneous activity.** The activity platforms affixed under the experimental chambers were used to concurrently record both the animals’ large and small movements throughout each 190-min DRL session. Almost immediately (3–5 s) after the animal was placed inside the experimental chamber, the DRL program, and then the Threshold activity program, were started. Both the DRL performance and activity were recorded daily throughout the duration of the experiment.

**Cocaine cumulative-dosing regimen.** Animals had been habituated with the procedure of cumulative oral saline injections because of their previous drug history as described above. Twenty-seven days (range: 18–32) after the last drug treatment, animals received a cumulative 0.9% saline-dosing series. That is, an injection was given during each time-out period preceding each of the five sub sessions comprising a DRL session. Then, one increasing cumulative cocaine dose regimen was given during a session; each subsession was preceded by a cocaine dose: 1, 2, 7, 20, and 45 mg/kg, which produced five cumulative doses of cocaine (1–75 mg/kg). A period of 7 to 12 days separated the saline gavage dosing from the increasing cumulative cocaine dosing.

The pre-exposure to d-amphetamine 18 to 32 days before cocaine administration likely did not alter cocaine’s PK based on the metabolic pathways for the two drugs. Amphetamine is metabolized by cytochrome P450 CYP2D1 in rats (Law and Moody, 1994), whereas cocaine is mainly (90%) metabolized by nonspecific plasma and tissue esterases, along with some involvement of cytochrome P450 3A enzymes (Kanel et al., 1990). Thus, any alteration (e.g., enzyme induction) in cytochrome P450 systems by d-amphetamine would have minimal effects on cocaine metabolism.

**Data Analyses.** The IRT distributions and spontaneous activity were analyzed after administration of the vehicle and cocaine cumulative doses for the five consecutive 35-min sub sessions. Baselines of both behaviors for each session immediately preceding an injection were also analyzed. Thus, for each rat, there were two baseline-day values for each behavior that were averaged and treated as the mean baseline value. The total number of responses consisted of responses with IRTs ≥45 s and <45 s, which are the reinforced and nonreinforced responses, respectively. Behavioral parameters were derived from the IRT distributions and were calculated as rate (responses/min): the shorter response rate, density of reinforcement, and total response rate. Specifically, the shorter response rate is defined as the responses per minute with IRTs <45 s and density of reinforcement (responses with IRTs ≥45 s) expressed in reinforcements per minute. Efficiency was calculated as the ratio of number of reinforced responses to the total responses. In past research, we have used the density of reinforcement in the 45- to 55-s bin to characterize drug actions (Lau and Heatherington, 1997; Lau et al., 1997, 1999a); thus, in the present study, we analyzed the IRTs in the 45- to 55-s bin to facilitate comparison with our previous work. Hereafter, the term “density of reinforcement” refers to the density of reinforcement in the 45- to 55-s bin. For spontaneous activity, both large and small movements were analyzed.

**Statistical Analysis.** One- and two-way repeated-measures (RM) ANOVAs followed by Newman-Keuls tests using SigmaStat (SPSS Inc., Chicago, IL) were performed as appropriate.

**PK-PD Modeling**

We used a between-group design for the PK-PD modeling to prevent any effect of blood sampling on behaviors as was done in our previous studies (Lau and Heatherington, 1997; Lau et al., 1997,
PK Analysis. The cocaine serum CTP after i.v. bolus administration is described by an open two-compartment model, with elimination from the central compartment (Fig. 1). The compartmental model parameters, the volume of distribution for the central compartment ($V_c$), and intercompartmental rate constants ($k_{12}, k_{21}$) are used to calculate the parameters in the equation, $C_t = Ae^{-at} + Be^{-bt}$, using standard formulae, where the terms $A$ and $B$ represent the extrapolated zero intercepts, and $a$ and $b$ represent the apparent first order distribution and elimination rate constants, respectively. For the oral route of administration an absorption rate constant, $k_a$, was also calculated. The PK parameters clearance (Cl) and volume of distribution at steady state ($V_\text{ss}$) were calculated using standard noncompartmental methodology. The area under the serum cocaine concentration-time curve from time 0 to infinity [AUC$_{0-\infty}$] and the area under the first moment of the serum cocaine concentration-time curve [AUMC$_{0-\infty}$] were obtained from the SAAM II software system. The absolute oral bioavailability (F) can be estimated from the PK model. We analyzed the i.v. and p.o. bolus cocaine profiles simultaneously, assuming the distribution and elimination characteristics were the same for a subject regardless of the route of administration except for the absorption phase as performed in our previous studies (Ma et al., 1999a; Wang et al., 1999). According to the basic principles of multiple dosing (Gibaldi and Perrier, 1982), a cocaine CTP for the escalating cumulative-dose regimen was simulated using the parameter values (Table 1) and inputting dose sizes (1, 2, 7, 20, and 45 mg/kg) and $\tau$ (35 min) into the SAAM II software. Alternatively, cocaine CTP also can be calculated by using standard equations (Gibaldi and Perrier, 1982).

**Pharmacodynamic Model**

![Fig. 1 Simultaneous optimization of cocaine CTP (n = 4; s1–s4) simulated from PK parameters with effect-time profiles of shorter response rate, large movements, and density of reinforcement (n = 8; s1–s8) using the three PD models for the escalating cumulative-dose regimen (1–75 mg/kg).](image-url)

**Table 1**

Cocaine PK parameters (CV%) estimated by simultaneous optimization of serum cocaine CTPs after i.v. (2 mg/kg) and p.o. (20 and 40 mg/kg) administration

<table>
<thead>
<tr>
<th>i.v. Cocaine</th>
<th>p.o. Cocaine</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_c$ (L/kg)</td>
<td>1.98 (8.18)</td>
</tr>
<tr>
<td>$V_{ss}$ (L/kg)</td>
<td>4.00 (3.69)</td>
</tr>
<tr>
<td>Cl (L/h/kg)</td>
<td>10.3 (1.83)</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>23.4 (1.87)</td>
</tr>
</tbody>
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**PD Models.** The sigmoid $E_{\text{max}}$ and the classical inhibitory $E_{\text{max}}$ models have been used to describe and predict the effects of i.v. cocaine on locomotor activity and operant behavior (Lau et al., 1999a,b; Ma et al., 1999a). The increases in shorter response rate or large movements were described by the sigmoid $E_{\text{max}}$ model (Holford and Sheiner, 1982) according to the following equation:

$$ E_{\text{max}} = E_0 + \frac{E_{\text{max}}C^n}{EC_{50}^n + C^n} $$

where $E_{\text{max}}/E_0$, $C$, $E_{\text{max}}$ and $EC_{50}$ are the effect at concentration $C$ within the central compartment, the effect in the absence of cocaine, the maximal effect, and the concentration required to produce 50% maximal effect, respectively, and $n$ is the Hill factor. The subscripts “srr” and “lm” denote shorter response rate and large movements, respectively. The decrease in density of reinforcement was described by the inhibitory sigmoid $E_{\text{max}}$ model (Dayneka et al., 1993) according to the following equation:

$$ E_{\text{dr}} = E_0 \left[1 - \frac{C}{C^n + IC_{50}}\right] $$

where $E_{\text{dr}}$, $C$, $E_0$, and $IC_{50}$ are the effect at concentration $C$ within the central compartment, the effect in the absence of cocaine, and the cocaine concentration required to produce 50% inhibition, respectively, and $i$ is the Hill factor. The subscript “dr” denotes density of reinforcement.

**Integration of PK and PD.** Because effects of cumulative cocaine dosing on small movements were largely not statistically significant (see below; Fig. 8B), no attempt was made to estimate the PD parameters for this behavioral measure. The simulated CTPs for the escalating oral cocaine dosing were integrated with the effect-time profiles of the shorter response rate, large movements, and density of reinforcement using the respective PD models for the estimation of the pertinent PD parameters. All data were fit simultaneously; only parameters resulting from the integrated model are presented (Fig. 1).

**Results**

**PK of Cocaine and Its Metabolites**

Figure 2, A to C, shows the mean serum cocaine (filled circles) and its metabolite (open symbols; benzoylecgonine and norcocaine) CTPs for the i.v. 2 mg/kg and the two oral doses (20 and 40 mg/kg), respectively. The PK parameters were estimated by simultaneous optimization of the i.v. dose
with both oral doses (Table 1); these PK values were similar to those estimated by simultaneous optimization of the i.v. dose with either of the two oral doses (data not shown). Based on these results, cocaine disposition appears not to be dose dependent in the dose range examined. Cocaine decayed biexponentially after i.v. administration, with an initial half-life ($t_{1/2a}$) of 3.6 min, and a terminal half-life ($t_{1/2b}$) of 22.8 min. The observed individual animal serum cocaine CTPs for the i.v. dose shown in Fig. 2D have less between-subject variability compared with those for the two p.o. doses (20 and 40 mg/kg) as shown in Fig. 2, E to F. Therefore, the predicted cocaine CTP for the i.v. dose more closely approximates the mean CTP, whereas the predicted CTPs for the two oral doses were somewhat lower than those of the mean values. The PK modeling was performed with different weightings and the best fit was achieved with a weighting of $1/(0.1^2y)$, where $y$ is the predicted concentration.

The mean benzoylecgonine concentrations peaked after 20 min after administration of i.v. and p.o. cocaine doses, and remained relatively high for the duration of the blood sampling regardless of routes of administration. Although norcocaine CTP was similar in shape to that of the parent compound for each route of administration, the formation of norcocaine after administration of the i.v. cocaine dose was significantly lower than that of the parent compound as judged by a two-way RM ANOVA ($P < .01$). In contrast, for the two p.o. doses, norcocaine CTPs did not differ from those of cocaine ($P > .05$).

The mean serum CTPs of cocaine and its two metabolites after administration of the five oral cumulative doses of cocaine (1–75 mg/kg) are shown in Fig. 3A. Cocaine and its two metabolites were detected in serum within 15 min after administration of the first dose (1 mg/kg). In general, cocaine and norcocaine concentrations increased across the five cocaine doses; however, both drug concentrations after 35 min post injection of the fourth and fifth cocaine doses were somewhat lower than those of the respective 15-min time point data. In contrast, benzoylecgonine concentrations remained high for the duration of the blood sampling. A two-way RM ANOVA revealed that the serum norcocaine CTP did not differ from that of cocaine ($P > .05$), although norcocaine concentration at 15 min after administration of the fifth cocaine dose was significantly lower than the cocaine concentration ($P < .05$).

Figure 3B shows that the cocaine CTP (solid line) simulated from the SAAM II software system for the cumulative cocaine dosing regimen using the PK parameters (Table 1), dose sizes, and $\tau$. Cocaine concentrations increased, reached a maximum (peak, $C_{\text{max}}$), and began to decline to a minimum (trough, $C_{\text{min}}$) before a higher cocaine dose was administered. Consequently, cocaine concentrations resulting from the sub-
sequent increasing doses were progressively higher. The observed CTPs of the pooled data for the four rats (filled symbols) were described well by the predicted CTPs (line). In light of this, we simulated cocaine CTPs for other multiple-dose regimens using the PK parameter values as representative CTPs for comparison. Figure 3, C and D, show cocaine CTPs after administration of five equal doses (5–40 mg/kg) separated by 35-min intervals and of five equal doses (10 mg/kg) separated by either 15- or 35-min intervals, respectively. Collectively, the steady-state concentrations ($C_{ss}$) are reached approximately at 90 min and thereafter oscillate between maximum ($C_{ss,max}$) and minimum ($C_{ss,min}$) concentrations in the simulated CTPs for all the five equal doses regardless of dose size and $t$. In addition, the shorter the $t$, the higher the $C_{ss}$ (Fig. 3D).

**PD: DRL 45-s Performance and Spontaneous Activity**

**DRL 45-s Performance.** Figure 4A shows that the effects on IRT distributions of the five consecutive vehicle injections, one at the beginning of each time-out component (3 min), exhibit a function, which approximates a $g$-distribution with the peak occurring in the 45- to 49.9-s bin as judged by a two-way RM ANOVA $[F(12,84) = 37.88, P < .001]$. As shown in the figure, IRTs shorter than that marked by the first arrow ($<45$ s) are not reinforced; those greater than the point marked by the first arrow are reinforced ($\geq 45$ s), whereas IRTs between the two arrow markings specify the reinforced IRTs within the 45- to 55-s bin. Because IRT distributions did not differ significantly across the five subsessions, as shown by a two-way RM ANOVA $[F(4,28) = 0.79, P > .5]$, the mean value of the IRT distribution of the five subsessions for the vehicle injections was used (Fig. 4B) to facilitate the comparison between treatments. The mean effects of vehicle on IRT distribution also did not differ from baseline values; however, cocaine shifted the IRT distributions to the left in a dose-related fashion with the exception of the lowest dose (1 mg/kg). Thus, cocaine decreased the responses in the 45- to 55-s bin, whereas it increased the number of shorter IRTs (5–44.9 s) with dose. Nevertheless, the burst responses with IRTs $<1$ s remained unaffected regardless of dose size (0–75 mg/kg) by an one-way RM ANOVA $[F(4,28) = 1.03, P > .05]$. The four baseline performance indices (open circles) and the effects of saline dosing on the four measures (open squares) are shown as clustered points at the far left for the cumulative dose-response curves (Fig. 5, A–D). As seen in these figures, consecutive vehicle injections showed negligible effects on the four performance indices compared with the baselines ($P > .05$). In contrast, cocaine significantly decreased the density of reinforcement $[F(4,28) = 6.33, P < .001]$ and efficiency $[F(4,28) = 5.73, P < .005]$, whereas it increased the shorter response rate $[F(4,28) = 6.94, P < .001]$ and the total response rate $[F(4,28) = 4.56, P < .01]$ in a dose-related manner as reflected by two-way RM ANOVAs. It should be noted that cocaine doses of 1 and 3 mg/kg did not produce statistically significant effects on the four behavioral indices. However, one-way RM ANOVAs revealed that effects of the two higher doses (30–75 mg/kg) were significantly greater than those of saline injections for the four indices ($P < .005–.001$). Although

![Fig. 4. Mean effects of vehicle and cocaine administration on IRT distributions for each subsessions: vehicle (A) and cocaine (1–75 mg/kg) (B). All responses before the first arrow are nonreinforced (<45 s); others are reinforced ($\geq 45$ s). The region between the two arrows is the 45- to 55-s bin ($n = 8$).](image)

![Fig. 5. Mean (S.E.) effects of cocaine (1–75 mg/kg) on DRL performance: density of reinforcement (in reinforcements/min) (A); shorter response rate (in responses/min with IRTs <45 s) (B); total response rate (in responses/min) (C); and efficiency (D). Horizontal axis shows the cumulative cocaine dose during a session. B, baseline, and V, vehicle.](image)
effects of the 10-mg/kg dose on DRL performance did not differ from those of the 3-mg/kg dose, cocaine’s effects at this dose were deemed significantly greater than the respective saline injections at $P < .05$ level by one-way RM ANOVAs because of the smaller standard deviations for these data points.

The effects of saline injections on large or small movements were minimal ($P > .5$) when compared with those of baseline (Fig. 6, A and B). In contrast, cocaine significantly increased large movements ($F(4,28) = 7.76, P < .001$). Although the increases in large movements in doses ranging from 1 to 10 mg/kg were not deemed statistically significant, they increased linearly after the 10-mg/kg dose (Fig. 6A). However, the effects of cocaine on small movements remained relatively unchanged for all doses ($P > .05$).

Effect-time profiles for all the behavioral profiles were constructed for the cumulative cocaine dosing. The effects of vehicle were similar to baseline levels across subsessions except those measured at the first time point (i.e., 5 min) for each subsession, indicating placebo effects immediately after each saline injection, especially for the second and third subsessions (Figs. 7, A and B, and 8, A and B). The arrows denote time points where blood sampling occurred. Two-way RM ANOVAs revealed that the overall effects of cocaine at lower doses (1–3 mg/kg) on the shorter response rate, density of reinforcement, and large movements were not significantly different from those of saline injections or baseline values in the first two subsessions ($P > .05$); however, at higher doses (10–75 mg/kg), cocaine significantly altered these behavioral measures in the third, fourth, and fifth subsessions ($P < .005$). In contrast, no significant overall increases in small movements from the escalating cocaine administration were observed, regardless of dose size ($P > .05$); nevertheless, one-way RM ANOVAs revealed that cocaine did significantly increase small movements at 10- and 15-min time points during subsessions 4 and 5, respectively ($P < .05$). The specific significant disruptions by cocaine on all four measures are shown in Figs. 7 and 8.

Cocaine Concentration-Effect Relations for the Cumulative Dosing

Because PK parameter values estimated from data set 3 were derived from a broader dose range (20–40 mg/kg), we used these values to simulate cocaine CTP for the PK-PD analysis. Table 2 shows cocaine PD parameters estimated by the integration of simulated cocaine CTP with effect-time profiles of the shorter response rate, density of reinforcement, and large movements. Analysis of pooled data for the three PD models, as judged by AIC values, indicates that one set of PD parameters (i.e., $EC_{50}/IC_{50}$ and Hill factors) is more appropriate to describe concentration-effect relations across the three behavioral indices with the exception of the intrinsic differences in $E_{max}$ and $E_0$ values between the behavioral endpoints (AIC = 4.12) rather than either two (AIC = 4.20).
Discussion

Cocaine PK parameters characterized in this study using pooled data from dual-catheter implanted animals were within the same range as the mean PK parameters derived in our previous study using jugular-catheter animals (Ma et al., 1999a). Temporal changes in cocaine concentration across the five escalating doses were described and predicted from PK parameters, dose size, and τ (Fig. 3, A and B) and validated. Effect-time profiles for the four behavioral measures indicated that the complete dose-response relations from $E_0$ to $E_{\text{max}}$ were examined within the dose range used (Figs. 7 and 8). With the exception of the intrinsic differences in the values for $E_{\text{max}}$ and $E_0$ among the three behavioral measures, the concentration-effect relations could be characterized and predicted by one set of PD parameters (Table 2). Cocaine’s effects expressed as a function of serum concentrations (Fig. 9, A–C) are a more appropriate reflection than is the cumulatively administered dose size, which does not consider the extent of dose accumulation in the dosing procedure (Figs. 5 and 6); the former reflects the bioavailable dose determined by PK properties and/or the ongoing concentrations that include the accumulation of drug concentrations from preceding doses, even though the accumulation was not substantial with a $\tau$ of 1.5 half-life (35 min).

One set of PK parameters accounted for the two oral co-
caine doses (Table 1), suggesting that cocaine PK was dose independent in the dose range of 20 to 40 mg/kg; this was consistent with our results for i.v. cocaine (Lau et al., 1999a). Assessment of the linearity of cocaine’s PK using a within-subject design is a challenging task due to the restrictively short life of an implanted catheter; in our hands, the catheters generally remained patent for four to five dosing series. Both linear and nonlinear cocaine kinetics has been described (Barnett et al., 1981; Booze et al., 1997; Parker et al., 1998; Mets et al., 1999). Most dose-response cocaine PK studies used a between-subject design, with each rat receiving only a single dose (Booze et al., 1997; Mets et al., 1999). In light of the short catheter life, it is rational to characterize the PK of cocaine for the higher dose range (20 and 40 mg/kg) because dose-dependent kinetics was less likely to occur within the lower dose range once no apparent change occurred at higher doses. This assumption facilitates our simulation of cocaine CTPs for multiple dosing. Furthermore, the preponderant significant effects occurred in the higher dose range (Figs. 7 and 8). However, the extrapolation of linear PK from 20- and 40-mg/kg oral dosing to lower doses (1–7 mg/kg) remains to be investigated.

Examination of the concentration-effect relations elucidated cocaine’s differential effects on contingency-controlled versus unconditioned behavior (Lau et al., 1999a,b). Cocaine’s effects on fixed-ratio 70 and DRL 45-s performance were directly proportional to drug concentration; however, acute tolerance developed to the effects of cocaine on locomotor activity. In addition, cocaine effect-time profiles for shorter response rates more closely resembled those for locomotor activity (i.e., effects are immediate and shorter lived); the latter were qualitatively similar to the large movements measured in the present study. One set of PD parameters accounted for cocaine’s effects on the three behavioral indices, indicating that these effects are solely dependent on concentration regardless of dose. Specifically, acute tolerance did not develop to cocaine’s effects on large movements within an operant context, contrasting with cocaine’s effects on locomotor activity without an operant context (Lau et al., 1999b), implying that cocaine’s effects on general activity levels in humans also may be context dependent.

Cocaine is metabolized to its active metabolite, norcocaine (Hawks et al., 1974; Misra et al., 1974). The formation of norcocaine after cocaine administration makes it difficult to assess norcocaine’s contribution to the overall observed effects of cocaine administration in behavioral analysis. Nevertheless, it is important to evaluate the contribution of norcocaine to cocaine’s effects before making inferences about other PD mechanism(s) such as sensitization or tolerance. In previous studies, norcocaine was not detected after i.v. cocaine administration, whereas it was present for oral cocaine (Lau et al., 1999a; Ma et al., 1999a). In this study however, serum norcocaine concentrations were detected for i.v. cocaine using a more sensitive HPLC method, but the concentrations were low and transient (Fig. 2A). In contrast, serum norcocaine concentrations for the three oral-dosing series were as high as those of the parent compound (Figs. 2, B and C, and 3A). Thus, the formation of norcocaine was route and dose dependent. Oral cocaine was approximately three times more effective than i.v. cocaine in decreasing density of reinforcement as indicated by IC_{50} values under the DRL 45-s schedule (Ma et al., 1999a). One plausible explanation was the involvement of norcocaine in cocaine’s effects after oral administration. In this study, the IC_{50} value for density of reinforcement (0.012 µg/ml) more closely approximated that of oral cocaine (0.022 µg/ml; Ma et al., 1999a), indicating that norcocaine plays an important role in cocaine’s observed effects, especially after determining that norcocaine, when administered as a parent compound, was as potent as cocaine in decreasing density of reinforcement under the DRL 45-s schedule (Wang et al., 2000). Furthermore, the smaller IC_{50} value found in this study, compared with the previous study (Ma et al., 1999a), may be attributed to a greater contribution of norcocaine to cocaine’s effects, because norcocaine concentrations were higher and lasted longer (Fig. 3A). The integration of PK and PD provides an analytical methodology to partition drug effects into PK and PD components for a better understanding of the mechanism(s) involved in drug action regardless of dose regimen used. A manuscript in preparation compares norcocaine’s role in affecting behavior as a parent compound and as an active metabolite by PK and PK-PD analyses.

Although cocaine’s effects on behavior have been widely studied using cumulative dosing (Kleven and Woolverton, 1996; Schechter, 1997; Rowlett and Spealman, 1998), concentration-effect relations are lacking with the exception of a study conducted in monkeys (Lamas et al., 1995). In the present study, concentration-effect relations were characterized and predicted by cocaine concentrations with PD models for cumulative dosing once the cocaine CTP was defined pharmacokinetically. The proposed PK model allows one to not only simulate various cocaine CTPs but also to design optimal multiple-dose regimens for maintaining a desired behavioral C_{ss} concentration. We simulated a few CTPs for multiple-dosing regimens of common interest to behavioral pharmacologists for drug evaluations (Fig. 3, C and D). Two of the dose regimens have been used to investigate oral cocaine’s effects under the DRL 45-s schedule (Lobarinas et al., 1999). Although the time required to reach the C_{ss} level is generally a complex function of several PK parameters with multiple dosing, usually about 90% of C_{ss} will be reached within approximately four half-lives (Gibaldi and Perrier, 1982). Thus, cocaine C_{ss} levels are reached at approximately 90 min and thereafter oscillate between C_{ss,max} and C_{ss,min} concentrations in the simulated CTPs for all the five equal doses regardless of dose size and τ (Fig. 3C). To state this in PK-PD terms, the effects of cocaine should progressively increase across the first three subsessions and reach steady state in the fourth and fifth subsessions, which correspond with our previous results (Lobarinas et al., 1999). In addition, it is known in drug PK that the shorter the τ, the higher the C_{ss} level (Fig. 3D). This multiple, equal-dose regimen provides an ideal mode to study phenomena such as sensitization and tolerance by maintaining serum drug concentrations at C_{ss} levels to investigate how behavioral effects deviate from effects expected at C_{ss} levels, contrasting with the progressive increases in C_{max} and C_{min} levels for the escalating cumulative-dose regimen (Fig. 3, A and B).

In summary, the cocaine CTP after a single-dose-administration procedure differed from the CTP after the corresponding dose within a cumulative-dosing procedure as shown in this (Figs. 2E and 3B) and other studies (Lamas et al., 1995); thus, consideration of PK factors is important when comparing the effects of a specific dose across the two procedures.
The difference in drug PK profiles may explain why the observed effects for the two procedures on some occasions were quantitatively identical (Wenger, 1980; Terry, 1992; Schechter, 1997), whereas on other occasions were qualitatively similar but quantitatively different (Thompson et al., 1983). The effects were most likely to be similar for the two dosing procedures when the comparisons were made in a dose range that produces maximal effects (Wenger, 1980; Spealman et al., 1989; Lamas et al., 1995). That both plasma and brain cocaine concentrations increased after chronic administration in animals (Misra et al., 1976; Nayak et al., 1976; Reith et al., 1987; Pettit et al., 1990) may account for sensitization observed after repetitive-dose regimens (Terry, 1992; Lobarinas et al., 1999). Again, it is crucial to determine parallel PK profiles in these studies before other mechanisms can be inferred. The proposed oral cocaine PK model can be applied to animals of the same species, age, gender, and food regimen to simulate rational cumulative or multiple dose regimens by varying dose size and/or \( t \). Additionally, concurrent monitoring of operant and locomotor activity behavior within conditions in which such an environment provides a novel behavioral paradigm to investigate drug effects on spontaneous activity under conditions in which a behavioral contingency exists.

References


