

# Pharmacokinetics of Cocaine in Maternal and Fetal Rhesus Monkeys at Mid-Gestation

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## ABSTRACT

We compared pharmacokinetics of cocaine and its metabolite, benzoylecgonine, in pregnant rhesus monkeys and their fetuses at mid-gestation: 1) after a single intravenous dose of cocaine, 2) after a single oral dose of cocaine, 3) after the last oral cocaine administration of a 50-day-long chronic cocaine treatment, and 4) on the last day of a 50-day-long chronic treatment with five daily intravenous cocaine injections. We found that intravenous administrations of cocaine produced maximal maternal levels of benzoylecgonine below the plasma levels for cocaine. In contrast, oral administrations resulted in the maximal maternal plasma levels of this metabolite significantly above those of cocaine. The bioavailability of the orally

administered cocaine was calculated as 25%. Cocaine was detectable in the fetal plasma at maximal levels of approximately 1/5 of peak maternal levels for both single intravenous and single oral administrations. The maximal plasma levels of benzoylecgonine for the fetuses of the intravenously treated mothers were close to those of cocaine, whereas peak levels of this metabolite in the plasma of the fetuses of the mothers receiving the oral treatments were above those of cocaine. The chronic treatments resulted in significantly higher maximal levels of cocaine in the fetal circulation compared with those produced by single drug administrations.

Cocaine abuse during pregnancy continues to be a serious problem in the inner cities of the United States (Dudish and Hatsukami, 1996; Richardson et al., 1999; Scher et al., 2000). Consequently, there is a need to understand the pharmacokinetics of cocaine in pregnant mothers and their fetuses. For obvious reasons, the data on transplacental pharmacokinetics of cocaine in humans are very limited and confined to a few post-mortem case reports (Mittleman et al., 1989; Apple and Roe, 1990; Klein et al., 1992). Also, little is known about cocaine pharmacokinetics in fetuses of other primates. We were only able to find a single report describing the changes in the maternal/fetal concentration of cocaine injected intramuscularly to anesthetized near term macaque monkeys (Binienda et al., 1993).

Over the last several years, we have been involved in the examination of the consequences of the prenatal cocaine exposure in rhesus monkeys born from mothers receiving oral drug treatment at mid-gestation (Lidow, 1995, 1998; Lidow and Song, 2001; Lidow et al., 2001). It has been demonstrated that oral cocaine administration can serve as a good laboratory model of cocaine snorting (Van Dyke et al., 1977; Wilkinson et al., 1980; Jufer et al., 1998). Indeed, administration of similar doses of cocaine by each of these routes results in virtually identical levels of cocaine in plasma (Van Dyke et

al., 1977; Wilkinson et al., 1980; Fattinger et al., 2000). The kinetics of postpeak decline in plasma cocaine levels are also the same for both routes of administration (Van Dyke et al., 1977; Wilkinson et al., 1980). Finally, both intranasal and oral cocaine administrations produce relatively high blood levels of cocaine metabolites (Cone et al., 1994; Jufer et al., 1998). This is due to the fact that a significant portion of the snorted cocaine reaches the gastrointestinal tract and, thus, is processed by the organism as an orally administered drug (Cone et al., 1994; Fattinger et al., 2000). The only difference in the pharmacokinetics of cocaine taken by the intranasal and oral routes is that in the case of the former route of administration the peak plasma levels of this drug occur about 30 min earlier than in the case of the administration by the latter route (Inaba, 1989). Our studies revealed that cocaine administered to pregnant monkeys in accordance with our model can induce significant alterations in cerebral cortical development (Lidow, 1995, 1998; He et al., 1999; Lidow and Song, 2001; Lidow et al., 2001).

The present article describes the maternal and fetal pharmacokinetics of cocaine and its major metabolite, benzoylecgonine, during the oral administration of cocaine to mid-term pregnant monkeys used in our model of prenatal drug exposure. A separate analysis was performed for a single oral dose and the last dose of cocaine in the chronic oral daily drug treatment. We also compared the pharmacokinetic

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**ABBREVIATIONS:** AUC, area under the plasma drug concentration-time curve; MRT, mean residence time.

ics of cocaine given orally and by a single intravenous injection. In addition, our study included analysis of the pharmacokinetics of cocaine administered by multiple daily intravenous cocaine injections. The latter mode of the treatment was designed 1) to provide for pharmacokinetics of cocaine close to those produced by smoking of crack (Jeffcoat et al., 1989; Isenschmit et al., 1992; Cone et al., 1994; Cone, 1995), which is the most prevalent form of cocaine administration today (Hays et al., 1999; Richardson et al., 1999; Scher et al., 2000); and 2) to replicate the average pattern of cocaine abuse by female drug addicts (Gossop et al., 1994; Richardson and Day, 1998).

## Materials and Methods

**Animals.** Eight healthy time-pregnant rhesus monkeys (*Macaca mulatta*), 5 to 7 years of age, were purchased from the commercial sources. The monkeys arrived at the University of Maryland Animal Facilities between pregnancy days 25 and 30 (E25–E30). Throughout the study, the animals were housed in individual cages and were fed High Protein Monkey Chow (Ralston Purina Co., St. Louis, MO), with fresh fruits given twice a day. The water was available at libitum. The day following their arrival, the animals were sedated with ketamine and custom-fitted in nylon vests to accustom them to this protective gear. Two of the monkeys were sedated again at E40, and prepared for chronic intravenous administration of cocaine as described in Mello et al. (1993a,b). This involved the surgical implantation of a silicone catheter in the jugular vein. The catheter was exited at the mid-scapular region under protection of the aforementioned nylon vests. Upon return of the animal to its cage, the catheter was connected to flexible stainless steel cables and fluid swivels, which allowed for a free movement within the cage. Cocaine hydrochloride (Research Technology Branch, National Institute of Drug Abuse, Rockville, MD) was administered beginning on E41 with five daily injections of 1 mg/kg each at 8:00 AM, 9:00 AM, 10:00 AM, 11:00 AM, and 12:00 PM. The drug was injected in 3 ml of sterile saline within a period of 40 to 50 s. The dosing of cocaine was based on the amount of this drug self-administered daily by an average pregnant crack cocaine user during the second trimester of pregnancy (Richardson and Day, 1998; Richardson et al., 1999) and corrected for the bioavailability of smoked cocaine base (Cone, 1995). The number of daily injections was equivalent to the average number of cocaine administrations reported for female drug addicts during pregnancy (Gossop et al., 1994; Richardson and Day, 1998). The interval between administrations was similar to that used in previous human models of cocaine abuse (Jufer et al., 1998). Also beginning on E41, two additional pregnant monkeys were treated with cocaine orally administered (in fruit treats) at a dose of 10 mg/kg, twice a day, at 8:00 AM and 8:00 PM. This regimen was the same as the one used in our previous studies of the consequences of prenatal cocaine exposure (Lidow, 1995; He et al., 1999; Lidow and Song, 2001; Lidow et al., 2001). The remaining four animals received no chronic cocaine treatment. They were used for the examination of the pharmacokinetics of single oral and intravenous doses of cocaine administered on the day of the blood collection (see below).

On the afternoon of E89, all animals were sedated with ketamine and placed under isoflurane anesthesia. Two animals were implanted with silicon catheters into the jugular vein for a single cocaine injection. In addition, each animal was implanted with a silicon catheter into the femoral vein to collect maternal blood samples. All catheters were tunneled subcutaneously to the mid-scapular region. Subsequently, the catheters for collection of fetal blood were implanted in all animals as described by Dickinson et al. (1980) and Ho et al. (1993). For this purpose, a transverse incision was made through the abdominal wall and uterus to expose the fetus and placenta. Amniotic fluid was collected to reduce the uterine pressure. It was returned to the amniotic cavity at the conclusion of the

surgery. The fetal head was exteriorized to the mandibulum, and allis tissue forceps were used to attach the fetal skin to the uterine incision. A parapharyngeal incision was made in the fetal neck, and a polyvinyl catheter was inserted into the internal jugular vein. The catheter was anchored to the fetal skin closure, and the fetus was returned to the uterus. The abdomen was closed in layers and the catheter was tunneled to the middle-scapular region to join the other catheters. All animals were put in the tether system described above and allowed unrestrained recovery with the maintenance of daily cocaine treatments when appropriate. Immediately following the surgery, the animals received 25 mg/kg cefazolin i.m., 50 mg of iron dextran i.m., and 1.0 ml of vitamin B complex i.m. Ketofen (5 mg/kg), an analgesic, was administered i.m. approximately 1 h after the discontinuation of isoflurane anesthesia and repeated at 6-h intervals for 24 h. The treatment of animals in this study was approved by the University of Maryland Animal Care and Use Committee under the guidelines of the National Institutes of Health and Public Health Services.

**Blood Collection.** Blood collection for analysis was performed on E91, 2 days after the surgery to allow the animals to recuperate and to eliminate the possible effects of anesthetic and analgesic drugs on cocaine pharmacokinetics. In the two animals receiving daily oral cocaine treatments blood collections were performed at 1, 5, 10, 20, 30, 60, 120, 240, 360, 480, and 720 min following the morning drug administration. The same blood collection times were used for the two animals receiving a single morning oral administration of 10 mg/kg cocaine and the two animals receiving a single morning intravenous injection of 1 mg/kg cocaine. For the two animals receiving multiple daily intravenous cocaine injections, blood collections were performed at 0.1, 1.1, 2.1, 2.5, 3.1, 3.5, 4.1, 4.5, 5.5, 6.0, 8.0, and 12.0 h after the first injection of the day. During each sampling, 300  $\mu$ l of maternal blood and 150  $\mu$ l of fetal blood were collected in heparinized Vacutainer tubes containing NaF and acetic acid. The samples were centrifuged at 5000 rpm for 15 min at 4°C. The supernatant was collected and stored at –70°C.

**Analysis of Concentrations of Cocaine and Benzoylcegonine.** The samples were analyzed for cocaine and benzoylcegonine according to the methodology of Cone et al. (1994) and Wang et al. (1994), with slight modifications. Specimens were mixed with internal standard solutions [trideuterated analogs of cocaine and benzoylcegonine (Sigma Chemical Co., St. Louis, MO)], diluted with acetate buffer (pH 6), filtered through fritted 9RFV02F4P reservoirs (United Chemical Technologies Co., Bristol, PA), and extracted by solid-phase extraction using Clean Screen DAU, 200 mg-10-ml filtration columns (United Chemical Technologies Co., Bristol, PA). Cocaine and benzoylcegonine were eluted with freshly prepared solvent (methylene chloride/2-propanol/ammonium hydroxide, 80:20:2, v/v/v). The eluent was evaporated under nitrogen in a 40°C water bath and reconstituted in 20  $\mu$ l of acetonitrile. The samples were then incubated for 30 min at 80°C with 20  $\mu$ l of derivatized reagent [*N,O*-bis-(trimethylsilyl)trifluoroacetamine, containing 1% trimethylchlorosilane]. Chromatography-mass spectrometry was performed using 1- $\mu$ l aliquots of the derivatized extract. The analysis was performed on a Hewlett-Packard 5971 mass selective detector interfaced with a Hewlett-Packard 5890A gas chromatograph (Hewlett-Packard, Meriden, CT). The splitless injection mode with purge-off time of 0.5 min was used for all analyses. Ultrapure grade helium was used as the carrier gas at a flow rate of 1 ml/min. The initial oven temperature was 70°C held for 1 min, programmed to 220°C at 35°C/min held for 0.25 min, programmed to 250°C at 10°C/min and held for 3 min. The injection port and transfer temperature were maintained at 250 and 280°C, respectively. The mass-selective detector was operated in the selected-ion monitoring mode. The ions for each compound were monitored in the following elution order (quantitative ion indicated in parentheses): [<sup>2</sup>H<sub>3</sub>]cocaine, *m/z* (185), 85; cocaine, *m/z* (182), 82, 303; and [<sup>2</sup>H<sub>3</sub>]benzoylcegonine, *m/z* (243), 85; benzoylcegonine, *m/z* (240), 82, 361. Quantification of cocaine and benzoylcegonine was based upon ratios of peak areas to the corre-

sponding deuterated internal standards. Duplicate matrix-matched calibration curves for both cocaine and benzoylecgonine were processed with each batch of specimens. Curves were constructed across the concentration range of 3 to 4000 ng/ml for both analytes. Control samples, containing the analytes at concentrations of 50, 250, and 1000 ng/ml, were also processed in duplicate with each run. The concentrations of cocaine and benzoylecgonine were calculated as micrograms per liter of plasma.

The collected data were analyzed with a PK Solutions software for Macintosh (Summit Research Co., Ashland, OH), which uses a non-compartmental method of pharmacokinetic analysis. The calculated parameters included:  $t_{max}$ , time to maximum plasma concentration;  $C_{max}$ , maximum plasma concentration;  $t_{1/2}$ , elimination half-life;  $K_{el}$ , elimination rate constant; AUC, area under the plasma drug concentration-time curve; and MRT, mean residence time corresponding to 63.2% residence in the body. For each of these parameters, the initial statistical analysis included a three-way ANOVA with the treatment (single i.v./single p.o./chronic p.o./chronic i.v.), age (mother/fetus), and chemical compound (cocaine/benzoylecgonine) as variables. This was followed by a Tukey's post hoc comparison between individual means (Prism; GraphPad Software, Inc., San Diego, CA). Since in this case ANOVA is essentially an obligatory prescreening of the data for Tukey's tests, the present article focuses on the latter tests under *Results*. Therefore, the  $p$  values presented were generated by Tukey's analysis. The differences were considered statistically significant at  $p < 0.05$ . The bioavailability of orally administered cocaine was calculated as follows:  $BIO = [AUC_{(p.o.)}/AUC_{(i.v.)}] \times [Dose_{(i.v.)}/Dose_{(p.o.)}] \times 100$ , where BIO is bioavailability,  $AUC_{(p.o.)}$  and  $Dose_{(p.o.)}$  are mean AUC and dose for a single oral cocaine administration, and  $AUC_{(i.v.)}$  and  $Dose_{(i.v.)}$  are mean AUC and dose for a single intravenous cocaine administration.

## Results

**Single Intravenous Cocaine Injection.** After a single intravenous injection of cocaine, the plasma levels of this drug in the pregnant rhesus monkeys reached maximum levels within 1 to 2 min (Fig. 1; Table 1) the fetus, the peak levels were reached 1 to 2 min later (Fig. 1; Table 1). The maximal fetal plasma levels of cocaine after a single intravenous injection were more than 5 times lower than the peak levels in the mothers ( $p = 0.005$ ; Fig. 1; Table 1). The AUC of the fetuses was also more than 4 times smaller than the maternal AUC ( $p = 0.006$ ; Table 1). The  $t_{1/2}$ ,  $K_{el}$ , and MRT values in the fetuses were comparable to those in the mothers ( $p > 0.05$ ; Table 1). In both mothers and fetuses, the levels of cocaine in blood declined to virtually undetectable levels within 8 h following the injection (Fig. 1).

The peak levels of benzoylecgonine in the plasma of the pregnant monkeys receiving a single intravenous cocaine injection were less than half the peak levels of cocaine ( $p = 0.025$ ; Fig. 1; Tables 1 and 2). These peak levels were attained approximately 1 h after cocaine administration. Benzoylecgonine had a relatively prolonged elimination half-life (Table 2) and was detectable at significant levels in the plasma even 12 h after cocaine administration (Fig. 1). Peak levels of this metabolite in the fetal circulation were reached within a time period comparable to that in the mothers (Table 2). However, the fetal peak values, were only 1/3 of those in the mothers ( $p = 0.047$ ; Fig. 1; Table 2) and were virtually equal to the maximal concentrations of cocaine in the fetal plasma ( $p = 0.231$ ; Fig. 1; Tables 1 and 2). The AUC of benzoylecgonine for the fetuses was nearly 4 times smaller than for the mothers ( $p = 0.027$ ; Table 2). It was also not significantly larger than the AUC of cocaine in the fetal

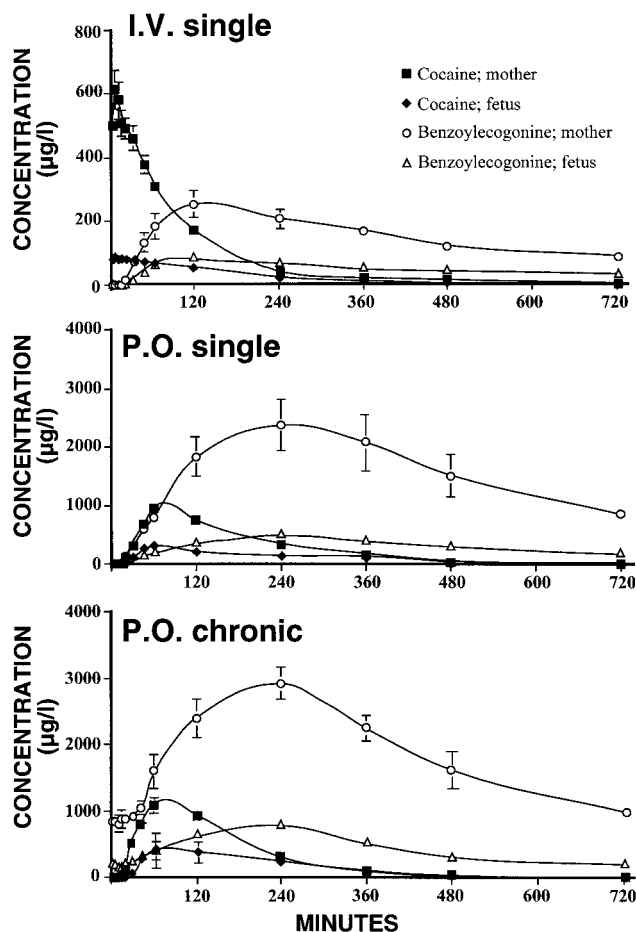


Fig. 1. Concentration of cocaine and benzoylecgonine in the mid-gestation pregnant rhesus monkeys and their fetuses after a single intravenous dose of cocaine (1 mg/kg), a single oral dose of cocaine (10 mg/kg), and the last dose of the 50-day-long chronic oral cocaine administration (10 mg/kg per administration). Each point represents a mean of  $n = 2 \pm$  S.D.

plasma ( $p = 0.120$ ; Tables 1 and 2). Detectable levels of benzoylecgonine were present in the fetal circulation even 12 h after the cocaine injection (Fig. 1).

**Single p.o. Cocaine Administration.** The maximal levels of cocaine in the circulation of pregnant monkeys after a single oral administration were reached within about an hour (Fig. 1; Table 1). Cocaine levels in plasma then declined with  $t_{1/2}$  values comparable to those seen in the animals receiving a single intravenous injection of the drug (Table 1). As in the latter animals, the cocaine levels were below detection by 8 h following administration (Fig. 1). The bioavailability of orally administered cocaine was calculated as 25% (Table 1). In the fetuses of the monkeys receiving a single oral administration of cocaine, the peak levels of this drug were reached within 15 to 30 min after such levels were reached in the maternal circulation (Fig. 1; Table 1). These levels were nearly 5 times lower than those in the mothers ( $p = 0.004$ ; Fig. 1; Table 1). The fetal AUC was also nearly 4 times smaller than the maternal AUC ( $p = 0.006$ ; Table 1). There were no significant differences between mothers and fetuses in the values of  $t_{1/2}$ ,  $K_{el}$ , and MRT of cocaine ( $p > 0.05$ ; Table 1). These values were also similar to those in the animals receiving a single intravenous injection of the drug ( $p > 0.05$ ; Table 1).

TABLE 1

Pharmacokinetic parameters of cocaine in mid-gestation pregnant rhesus monkeys and in their fetuses after a single intravenous dose of cocaine (1 mg/kg), a single oral dose of cocaine (10 mg/kg), and the last administration of the 50-day-long chronic oral cocaine treatment (10 mg/kg per administration)

The values are means of  $n = 2 \pm$  S.D.

Cocaine Administration	Animals	$t_{\max}$	$C_{\max}$	$t_{1/2}$	$K_{el}$	AUC	MRT	Bioavailability
		min	$\mu\text{g/l}$	h	l/h	$\mu\text{g/l/h}$	h	%
i.v. single	Mother	2.2 $\pm$ 1.0	520 $\pm$ 43	0.96 $\pm$ 0.3	0.72 $\pm$ 0.29	930 $\pm$ 77	1.5 $\pm$ 0.3	
	Fetus	4.9 $\pm$ 0.9	87 $\pm$ 7*	1.3 $\pm$ 0.5	0.53 $\pm$ 0.22	207 $\pm$ 16*	2.0 $\pm$ 0.4	
	Ratio: fetus/mother	2.22	0.17	1.35	0.73	0.22	1.30	
p.o. single	Mother	57 $\pm$ 12	980 $\pm$ 52	1.1 $\pm$ 0.4	0.63 $\pm$ 0.20	2311 $\pm$ 290	2.3 $\pm$ 0.5	25
	Fetus	76 $\pm$ 20	207 $\pm$ 39*	1.2 $\pm$ 0.3	0.57 $\pm$ 0.33	540 $\pm$ 105*	2.6 $\pm$ 0.4	
	Ratio: fetus/mother	1.33	0.21	1.09	0.90	0.23	1.13	
p.o. chronic	Mother	55 $\pm$ 19	1207 $\pm$ 48	0.89 $\pm$ 0.2	0.77 $\pm$ 0.28	2901 $\pm$ 311	1.8 $\pm$ 0.5	
	Fetus	74 $\pm$ 20	387 $\pm$ 34*	0.98 $\pm$ 0.3	0.71 $\pm$ 0.30	882 $\pm$ 106*	2.0 $\pm$ 0.4	
	Ratio: fetus/mother	1.35	0.32	1.10	0.92	0.30	1.13	

Fetal values that differ statistically from the maternal values ( $p < 0.05$ ; Tukey's post hoc analysis) are marked by asterisks.

TABLE 2

Pharmacokinetic parameters of benzoylecgonine in mid-gestation pregnant rhesus monkeys and in their fetuses after a single intravenous dose of cocaine (1 mg/kg), a single oral dose of cocaine (10 mg/kg), and the last administration of the 50-day-long chronic oral cocaine treatment (10 mg/kg per administration). The values are means of  $n = 2 \pm$  S.D.

Cocaine Administration	Animals	$t_{\max}$	$C_{\max}$	$t_{1/2}$	$K_{el}$	AUC <sup>a</sup>	MRT
		h	$\mu\text{g/l}$	h	l/h	$\mu\text{g/l/h}$	h
i.v. single	Mother	1.7 $\pm$ 0.6	247 $\pm$ 45	5.2 $\pm$ 1.2	0.13 $\pm$ 0.7	1640 $\pm$ 272	7.8 $\pm$ 1.3
	Fetus	1.9 $\pm$ 0.7	78 $\pm$ 32*	6.6 $\pm$ 1.6	0.11 $\pm$ 0.5	433 $\pm$ 90*	9.9 $\pm$ 2.0
	Ratio: fetus/mother	1.12	0.31	1.57	0.85	0.26	1.27
p.o. single	Mother	2.9 $\pm$ 1.0	2672 $\pm$ 352	6.9 $\pm$ 1.4	0.10 $\pm$ 0.03	24826 $\pm$ 799	11.1 $\pm$ 2.6
	Fetus	3.5 $\pm$ 1.3	506 $\pm$ 61*	5.8 $\pm$ 0.9	0.12 $\pm$ 0.05	4475 $\pm$ 280*	9.9 $\pm$ 1.7
	Ratio: fetus/mother	1.33	0.19	0.84	1.2	0.18	0.89
p.o. chronic	Mother	2.6 $\pm$ 0.8	3050 $\pm$ 121	7.2 $\pm$ 1.9	0.10 $\pm$ 0.04	28209 $\pm$ 978	11.5 $\pm$ 3.3
	Fetus	3.2 $\pm$ 0.9	891 $\pm$ 78*	6.7 $\pm$ 1.1	0.10 $\pm$ 0.06	6930 $\pm$ 712*	11.0 $\pm$ 4.2
	Ratio: fetus/mother	1.23	0.29	0.93	0.78	0.25	0.96

<sup>a</sup> AUC<sup>0-12h</sup>.

Fetal values that differ statistically from the maternal values ( $p < 0.05$ ; Tukey's post hoc analysis) are marked by asterisks.

In contrast to the pregnant animals receiving a single intravenous cocaine injection, in which the peak plasma levels of benzoylecgonine were much lower than the peak levels of cocaine, the animals receiving a single oral administration of the drug displayed the peak levels of this metabolite that exceeded more than twice the maximal levels of the parent chemical compound ( $p = 0.021$ ; Fig. 1; Table 2). In the fetuses, the maximal levels of benzoylecgonine were nearly 5 times lower than in the mothers ( $p = 0.013$ ; Fig. 1; Table 2) and were reached within approximately half an hour after the peak levels of this metabolite were detectable in the maternal circulation (Fig. 1; Tables 1 and 2). The maximal levels of benzoylecgonine in the fetal plasma were also more than twice as high as such levels of cocaine ( $p = 0.046$ ; Figs. 1 and 2; Tables 1 and 2). The fetal AUC of benzoylecgonine was more than 5 times smaller than the maternal AUC ( $p = 0.001$ ; Table 2), but more than 8 times larger than the fetal AUC of cocaine ( $p = 0.002$ ; Tables 1 and 2). For both mothers and fetuses, the elimination half-life of benzoylecgonine was between 5 and 7 h (Table 2), and, therefore, detectable levels of these metabolites were still seen in their plasma 12 h after cocaine administration (Fig. 1).

**Chronic p.o. Cocaine Administration.** In the circulation of pregnant monkeys, the peak levels and AUC of cocaine after an oral dose concluding a 50-day-long chronic treatment was only slightly larger than those observed after a single oral administration of this drug ( $p > 0.05$ ; Fig. 1; Table 1). Also, as in the case of a single drug administration, the peak levels and daily AUC of cocaine for the chronically exposed

fetuses were several magnitudes lower than for their mothers ( $p = 0.003$  and  $0.013$  for peak levels and AUC, respectively; Fig. 1; Table 1). However, these parameters in the plasma of the fetuses chronically exposed to cocaine were nearly twice as large as in the fetuses subjected to only a single cocaine treatment ( $p = 0.039$  and  $0.047$  for peak levels and AUC, respectively; Table 1).

We detected no significant differences between chronically treated mothers and fetuses in the values of  $t_{1/2}$ ,  $K_{el}$ , and MRT for cocaine ( $p > 0.05$ ; Table 1). These values were also similar to those in the animals receiving a single oral administration of the drug ( $p > 0.05$ ; Table 1). In both chronically treated mothers and fetuses, the levels of cocaine in the circulation declined to undetectable levels by 8 h following administration.

Similar to those for cocaine, the peak levels and daily AUC of benzoylecgonine in the chronically exposed fetuses were several magnitudes lower than those in their mothers ( $p = 0.002$  for both peak levels and AUC; Fig. 1; Table 1). In addition, the peak levels and AUC of benzoylecgonine in the orally chronically treated pregnant monkeys were not significantly different from the ones in the animals receiving a single oral dose of cocaine ( $p > 0.05$ ; Fig. 1; Table 2). However, both of these parameters were significantly larger in the fetuses of the former monkeys than in the fetuses of the latter animals ( $p = 0.038$  and  $0.045$  for peak levels and AUC, respectively; Fig. 1; Table 2).

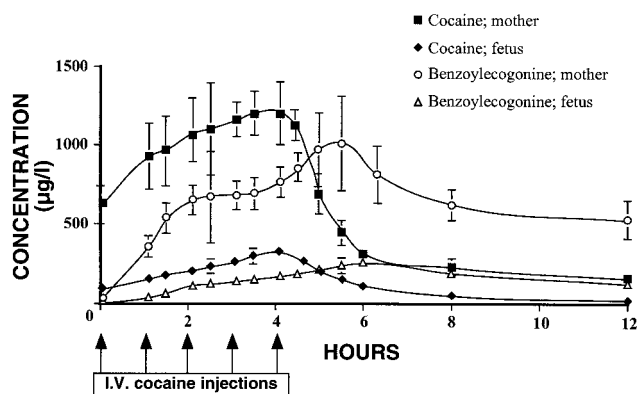
There were no significant differences between chronically treated mothers and fetuses in the values of  $t_{1/2}$ ,  $K_{el}$ , and

MRT of benzoylecgonine ( $p > 0.05$ ; Table 1). These values were also similar to those in the animals receiving a single oral administration of the drug ( $p > 0.05$ ; Table 1).

**Chronic Intravenous Cocaine Administration.** The peak levels of cocaine in plasma of pregnant monkeys subjected to five daily intravenous injections of cocaine for over 50 days were more than twice the peak levels of cocaine in pregnant animals receiving a single injection of this drug ( $p = 0.042$ ; Figs. 1 and 2; Tables 1 and 3). These levels were similar to the maximal levels of cocaine in the animals receiving chronic oral cocaine treatment ( $p = 0.086$ ; Figs. 1 and 2; Tables 1 and 3). The AUC of cocaine in the intravenously chronically treated monkeys, however, was more than twice as large as that for the morning cocaine administration in the animals chronically receiving the drug by the oral route ( $p = 0.026$ ; Figs. 1 and 2; Tables 1 and 3). This was because the multiple daily intravenous injections kept high plasma levels of cocaine 6 times longer than a single administration within the chronic oral treatment (Fig. 2). It should be remembered that in our studies cocaine was given orally twice daily. Consequently, the total daily AUC for the chronic oral treatment was much closer to that recorded for the chronic intravenous treatment.

The peak levels and AUC of cocaine in the circulation of pregnant monkeys receiving chronic intravenous treatment were significantly higher than these parameters in their fetuses ( $p = 0.027$  and  $0.010$  for peak levels and AUC, respectively; Fig. 2; Table 3). In addition, the maximal levels of cocaine in the plasma of fetuses of mothers receiving chronic intravenous treatments were nearly 4 times higher than those in the fetuses of mothers receiving a single drug injection ( $p = 0.020$ ; Figs. 1 and 2; Tables 1 and 3). These levels were close to the maximal levels of cocaine in the plasma of fetuses of mothers receiving chronic oral cocaine treatment ( $p = 0.103$ ; Figs. 1 and 2; Tables 1 and 3). However, the cocaine AUC in the plasma of the former fetuses was nearly twice as large as the AUC for the morning cocaine administration in the latter fetuses ( $p = 0.009$ ; Tables 2 and 3). This means that, after two daily oral treatments, the total daily AUC for the fetuses of mothers receiving cocaine orally was nearly twice as large as the AUC for the fetuses of the intravenously chronically treated mothers.

The peak plasma levels and AUC for benzoylecgonine in



**Fig. 2.** Concentration of cocaine and benzoylecgonine in the mid-gestation pregnant rhesus monkeys and their fetuses on the 50th day of chronic treatment with five daily intravenous injections of cocaine, 1 mg/kg each. The arrows mark the injection times. Each point represents a mean of  $n = 2 \pm$  S.D.

the mothers and fetuses subjected to the chronic intravenous cocaine treatment were several times smaller than those seen during the chronic oral treatment ( $p < 0.05$  in all cases; Tables 2 and 3). They were statistically indistinguishable from the ones recorded in mothers and fetuses of the animals receiving a single intravenous injection of cocaine ( $p > 0.05$ ; Tables 2 and 3).

## Discussion

This study supports the conclusion of previous reports (Binienda et al., 1993; Duhart et al., 1993; Saady et al., 1995) demonstrating a significant similarity between the pharmacokinetics of cocaine in nonhuman primates and human drug addicts. All the pharmacokinetic parameters calculated in the course of the present investigation were well within the range of these parameters obtained from human volunteers (Wilkinson et al., 1980; Barnett et al., 1981; Chow et al., 1985; Jeffcoat et al., 1989; Isenschmit et al., 1992; Cone et al., 1994; Cone, 1995). Even a relatively low bioavailability of the orally administered cocaine was close to that obtained in human studies of this route of drug administration (Wilkinson et al., 1980; Fattinger et al., 2000).

Our results also support the earlier observations of Binienda et al. (1993) that cocaine administered to pregnant rhesus monkeys can penetrate the placenta and enter the fetal circulation. Furthermore, both studies have demonstrated that the maximal levels of cocaine in the circulation of the fetuses are several magnitudes lower than the peak levels of this drug in their mothers. However, although we found the maternal/fetal ratio of peak cocaine levels after a single drug administration to be close to 1:5, this ratio was 1:7 in studies of Binienda et al. (1993). These differences may relate to the fact that, in contrast to the latter studies that examined cocaine pharmacokinetics in anesthetized near term animals, we used fully awake mid-term monkeys.

The comparison of the relationship between the maternal and fetal plasma levels of cocaine seen in the present study with those in the pregnant human drug abusers is rather difficult. The only human information in this regard comes from the post-mortem study of the pregnant victim of a car accident (Mittleman et al., 1989). In that case, the levels of cocaine in the fetal plasma were nine times lower than in the plasma of the mother. The authors of the report, however, speculate that such a low proportion of cocaine in the fetal circulation may be due to the fact that "the mother's death occurred so rapidly following the absorption of snorted drug that the pharmacokinetic compartment represented by the fetus did not reach equilibrium with the maternal blood" (Mittleman et al., 1989). The relationship observed in this study between the maximal levels of cocaine in the fetal and maternal circulation of rhesus monkeys was very similar to that reported for rodents and sheep (DeVane et al., 1989, 1991; Spear et al., 1989; Collins et al., 1999; Ma et al., 1999). This may indicate that this relationship is characteristic of all mammalian species.

As can be expected based on human studies (Jeffcoat et al., 1989; Cone et al., 1994; Jufer et al., 1998), the maximal levels of benzoylecgonine in a maternal plasma after the intravenous cocaine injection were much lower than those of cocaine, whereas the peak plasma levels of this metabolite after an oral cocaine administration were significantly higher than

TABLE 3

Plasma  $C_{max}$  and AUC for cocaine and benzoylecgonine in mid-gestation pregnant rhesus monkeys and in their fetuses on the 50th day of chronic cocaine treatment by five daily intravenous injections, 1 mg/kg each

The values are means of  $n = 2 \pm$  S.D.

Animals	Cocaine $C_{max}$ $\mu\text{g/l}$	Cocaine AUC $\mu\text{g/l/h}$	$C_{max}$ $\mu\text{g/l}$	Benzoylecgonine AUC <sup>a</sup> $\mu\text{g/l/h}$
Mother	1201 $\pm$ 201	5987 $\pm$ 641	998 $\pm$ 306	6390 $\pm$ 733
Fetus	326 $\pm$ 49*	1267 $\pm$ 219*	220 $\pm$ 57*	1370 $\pm$ 311*
Ratio: fetus/mother	0.27	0.21	0.22	0.18

<sup>a</sup> AUC<sup>0-12h</sup>.

Fetal values that differ statistically from the maternal values ( $p < 0.05$ ; Tukey's post hoc analysis) are marked by asterisks.

those of the parent chemical compound. The latter is due to the significant nonenzymatic hydrolysis of cocaine in the stomach and the nonenzymatic and enzymatic hydrolysis of this drug during the first pass metabolism (Cone et al., 1994; Sandberg et al., 1995; Jufer et al., 1998).

There is a long-standing controversy as to whether benzoylecgonine can cross the placental barrier. Although some investigators believe that the placenta is virtually impermeable to benzoylecgonine (Schama et al., 1998), others have demonstrated that this metabolite can cross the placenta in both humans and rodents, although to a much lower extent than cocaine (Simone et al., 1994; Sandberg et al., 1995; Morishima et al., 1997). Examinations of aborted human fetuses have shown significant plasma levels of benzoylecgonine (Apple and Roe, 1990; Klein et al., 1992). However, this may be a result of cocaine hydrolysis in the fetal circulation. The present study demonstrates the presence of benzoylecgonine in the fetal plasma after both intravenous and oral cocaine administration. We also found that, after an intravenous injection, the fetal peak levels of benzoylecgonine were close to the levels of cocaine. In contrast, the maximal levels of this metabolite were much higher than those of cocaine in the fetuses of the mothers receiving a single oral cocaine administration. This indicates that at least some of the benzoylecgonine in the latter fetuses came from the maternal circulation. It must be stressed that in all fetuses the plasma levels of benzoylecgonine were several times lower than in the maternal circulation, and even after chronic oral cocaine treatment they never reached levels capable of inducing cytotoxicity (Lin and Leskawa, 1994).

The final objective of the present study was to compare the cocaine and benzoylecgonine exposures in the fetuses of the mothers receiving chronic oral cocaine treatment (used in our previous analysis of the effects of this drug on cerebral cortical development; Lidow, 1995; He et al., 1999; Lidow and Song, 2001; Lidow et al., 2001) and the fetuses of the mothers subjected to a more realistic treatment by multiple daily intravenous cocaine injections. We found that in both cases the levels of maternal cocaine were within the range of those reported for human drug addicts (Jatlow, 1988; Jufer et al., 1998). We also found that chronic treatment with two daily administrations of 10 mg/kg cocaine produced somewhat more significant overall exposure of the fetuses to both cocaine and benzoylecgonine than the treatment with multiple daily intravenous injections of 1 mg/kg of the drug. On the other hand, the latter injections resulted in a much longer exposure of the fetuses to near maximal levels of cocaine.

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