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A Murine Model of Prenatal Cocaine Exposure: Effects on the Mother and the Fetus

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MIDDAUGH, L. D., W. O. BOGGAN, S. BINGEL, K. S. PATRICK AND W. XU. *A murine model of prenatal cocaine exposure: effects on the mother and the fetus.* PHARMACOL BIOCHEM BEHAV **55**(4) 565–574, 1996.—To develop and characterize a murine model for investigating the long-term effects of prenatal cocaine exposure, the present study established the route of drug administration and the doses to be used for pregnant C57BL/6 mice. Comparison of the effects of a high dose of cocaine (60 mg/kg) when gavaged or injected subcutaneously (SC) established patterns of pathology characteristic of administration route but no dominating logic for selecting one over the other route for prenatal studies; however, because of the fourfold greater brain levels, with no evidence of greater pathology, the SC route was selected. When injected daily during gestation days 12–18, the period of prenatal development of dopamine systems, cocaine at doses producing plasma concentrations consistent with its stimulatory effects reduced food ingestion and weight gains during pregnancy and fetal body and brain weights at term. The extent of these reductions was comparable to reports on babies exposed to cocaine prenatally. Furthermore, the present study suggests that maternal undernutrition is not a likely mediator of these perinatal effects and that differences in the amount of cocaine exposure may cause the contrasting effects of maternal cocaine noted in the human literature. **Copyright © 1996 Elsevier Science Inc.**

Prenatal cocaine C57BL/6J mice Cocaine pathology Intrauterine growth reduction

PRENATAL–maternal cocaine effects on the developing human fetus are generally confounded by polydrug use, nutritional deficiencies, and other life-style variables; however, such exposure reduces intrauterine growth and brain size as indexed by small head circumference (5,6,7,30,42,45,51,52). Although the body weight reduction appears to be transient, the reduction in head circumference extends to at least 3 years of age (43). A number of reports using rat models indicate that the birth weight of newborns is reduced by prenatal exposure to cocaine in the absence of other drugs. The body weight reductions appear to be transient, and relatively few of the reported studies have considered brain size [see review by Church et al. (9)]. In contrast to the numerous reports on the effects of cocaine on pregnant rats, there are few reports on the effects of maternal cocaine with mice as subjects; however, reductions in birth weight (11) and brain size (17) have been reported for this species.

As part of a research program to develop a murine model to investigate the long-term effects of prenatal cocaine exposure, the experiments reported here were done to determine if routes of administration and the doses used in previous studies on rats would be appropriate for pregnant C57BL/6 (C57) mice. Although the C57 mouse is reported to be less sensitive to some of the behavioral effects of cocaine than are some other inbred strains (27,46,49,50), they will actively work for cocaine as a reinforcer (16,20). Based on their more rapid acquisition of intravenous self-administration, C57 mice appear to be more sensitive than DBA mice to the reinforcing effects of cocaine, although the DBA mice exhibit a greater stimulatory effect (20). Previous reports have indicated that cocaine doses of 20 and 40 mg/kg administered subcutaneously (SC) to pregnant rats alter a number of behavioral parameters in the offspring (10,23,48). Some reports, however, have indi-

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cated that the SC route of cocaine administration produces a number adverse effects, particularly hair loss and necrotic open lesions surrounding the injection area (3,14) and that gavaged cocaine also produces a number of behavioral effects in the offspring (13). Accordingly, our initial experiment in C57 mice was to determine the influence of administration route, gavage (G) vs. SC injection, on body weight and several indices of pathology and on brain concentrations of cocaine in C57 mice.

The second experiment was to determine if the 20 and 40 mg/kg cocaine (HCl) doses used for rats were appropriate for our murine model with reference to other animal studies and to reports on human maternal cocaine use. In this experiment, we determined the effects of daily SC cocaine injections (20 or 40 mg/kg) during gestation days (GD) 12–18 on body weight, food ingestion and motor activity of the pregnant mice, on body and brain weights of the GD18 fetuses and on dopamine (DA) and dihydroxyphenylacetic acid (DOPAC) concentrations in nucleus accumbens and caudate of the dam and caudate of the fetuses. Cocaine concentrations were also examined in this experiment but were published elsewhere (35). The particular physical, behavioral and neurochemical measures taken were selected because of their frequent use in the literature concerning the effects of cocaine. GD12–18 was selected as the time of cocaine exposure because the overall aim of our research program is to assess the effects of prenatal cocaine exposure on DA systems throughout the life span. DA is first noted in mice on GD13 (19); therefore, the cocaine exposure occurred during the entire period of prenatal DA development.

Because the 40-mg/kg dose of cocaine used in Experiment 2 attenuated weight gain of the pregnant mouse, and body and brain weights of the fetus, a third experiment was conducted to examine the possible role of undernutrition in the effects of the high dose of cocaine on growth of the fetus.

METHODS

Subjects and Cocaine Treatment

Female C57 mice were purchased from Jackson Laboratories at 49 days of age. The animals were maintained in an AAALAC-accredited facility that was maintained at 21 ± 2°C and on a 12-h light:dark cycle (lights on at 0700 h). After a 2-week acclimatization period, cocaine administration was initiated for the mice in Experiment 1, and breeding procedures were initiated for Experiments 2 and 3. The mice were bred according to an established timed breeding procedure (34). Females were placed with males at 1700 h. The presence of a vaginal sperm plug the following morning defined the beginning of pregnancy (GD0). Mice with sperm plugs were weighed and caged individually. Pregnancy was confirmed via weight gain on GD11 (≥4.0 g), and the animals were assigned to drug-treatment conditions. Cocaine HCl was obtained from NIDA Drug Supply (Batch 7890-1022-131B, Research Triangle Institute, Research Triangle Park, NC).

Experimental Procedures

Experiment 1. In this experiment (Exp-1), female mice were administered cocaine (60 mg/kg as HCl, 0.01 ml/g body weight) once per day by either G (n = 6) or SC (n = 6) injection in the suprascapular region. One of the SC mice died due to causes unrelated to the treatment procedure. The treatments were continued for 7 days, during which body weight and general health conditions were monitored. Because our primary purpose was to evaluate potential differences that might arise due to route of administration and untreated mice would be unlikely to exhibit the pathology of interest, only two additional mice were used as saline controls. These mice received daily SC injections of saline. The mice were decapitated 30 min after the final cocaine administration, and brain tissue was collected to determine cocaine concentrations. Other tissues were collected for pathological examination (skin from the suprascapular region, heart, lung, kidney, gastrointestinal tract, liver, adrenal gland, bladder and uterus).

Experiment 2. The second experiment (Exp-2) contained three groups of pregnant mice assigned on the basis of cocaine dose. Beginning on GD12, the mice received daily SC injections (0.01 ml/g body weight) of saline (SAL; n = 15, pH 4.0) or cocaine (HCl) at 20 mg (C20; n = 13, pH 4.2) or 40 mg (C40; n = 16, pH 3.78) per kilogram of body weight between 0830 and 1000 h. All animals had food and water available ad libitum. There was no pair-fed saline group in this experiment. Body weights, food ingestion and water ingestion were determined prior to each daily injection through GD18. On GD18, the day prior to normal delivery, motor activity was assessed for 15 min beginning 10, 40, or 100 min after the final daily injection. Within 5 min of the activity test, the animals were killed, the fetuses were removed and weighed, then brains of the dams and fetuses were removed, weighed and frozen for subsequent assays. The general design for the experiment was a 3 (cocaine dose) × 3 (postinjection time) factorial. Although saline injections were not expected to alter either the behavioral or the neurochemical measures, the saline-injected mice were evenly distributed across the three postinjection times to control for the possibility of injection stress.

Experiment 3. The third experiment (Exp-3) was similar to Exp-2 and contained three groups of pregnant mice. The mice were injected with either saline or C40. One of the saline-injected groups (SAL; n = 7) had food and water available ad libitum as for Exp-2. An additional saline-injected group (SPF; n = 11) and the C40 group (n = 7) were given daily allotments of food and water based on the mean consumption by C40 mice of Exp-2. Again, weight gains of the dams during pregnancy and fetal body and brain weights on GD18 were determined. The design for this experiment was a univariate factorial with prenatal condition as the variable being tested (SAL, SPF, C40).

Behavioral Procedures

The effect of the cocaine doses on motor activity was determined in Exp-2 by using a Digiscan Animal Activity Monitor system with a two-animal option (Omnitech Electronics, Model RXYZCM(8) TAO, Columbus, OH). Three activity units, two chambers or quadrants per unit, were located in three 90- × 54- × 35-cm sound-attenuation boxes (1 unit per box). Each activity unit contained 16 photobeams positioned 5 cm apart; 8 on the x-axis and 8 on the y-axis. Photocells located on the wall directly opposite each photobeam were activated when the beam was interrupted. The Digiscan Analyzer recorded the beams interrupted and provided the distance (in centimeters) the animal traveled during testing as a measure of horizontal activity. Each unit was partitioned with acrylic into 20- × 20-cm quadrants. Mice were tested in one of two quadrants of each unit (i.e., 6 mice per test). The Digiscan Analyzer was interfaced with an IBM XT computer using ILAM software (Coulbourn Instruments, Lehigh Valley, PA). All testing was completed in a dark environment. Motor activity was recorded as total centimeters

traveled over the testing intervals described for each experiment. The chambers were cleaned of fecal matter and urine and wiped with disinfectant (50/50 solution of distilled water and alcohol) after each test to eliminate olfactory cues from previous runs.

Neurochemical and Analytical Procedures

Brain dissections. Caudates of fetuses were used for catecholamine assays; however, for the dams the brains were dissected to obtain tissue samples of both the caudate and accumbens. Frozen brains were placed ventral side up in an ice-cooled tissue slicer. Samples were taken from a 2.5-mm tissue slice rostral to the rostral border of the optic chiasm. The slice was placed on an ice-cooled glass plate in a drop of cold saline. The nucleus accumbens sample was obtained bilaterally from an area ventral to the lateral ventricle and dorsomedial to the anterior commissure with a 1.0-mm punch. The striatal sample was obtained bilaterally from an area bounded by the lateral ventricles medially and by the corpus callosum dorsolaterally with a 2.0-mm punch. Samples were flushed from the punch with 0.25 ml of the mobile phase (minus the sodium octyl sulfate) utilized for determination of DA and DOPAC.

DA and DOPAC concentrations. High pressure liquid chromatography (HPLC) with electrochemical detection (31) was used to determine DA and DOPAC content. Prior to homogenization, 0.75 ml of cold homogenate buffer was added to the vial containing the striatal tissue samples. The samples were homogenized with a sonifier and centrifuged at 18,000 rpm for 30 min in a DuPont Sorvall RC-5 Super-speed refrigerated centrifuge with an SS 34 rotor. The supernatant was transferred to a new vial and used for HPLC assay. The protein pellet was resuspended in 1.0 ml of 0.1 N NaOH and stored frozen until assay of protein content (2).

The HPLC system consisted of a Waters 510 pump, a C-18 precolumn, an Altex Ultrasphere-ODS reverse-phase column (5 μm × 4.6 mm inner diameter × 25 cm) kept at a constant 30°C, a Rheodyne 7125 injector with a 200μl loop, and an ESA Coulochem Electrochemical Detector (oxidation potential = +0.30). The mobile phase was essentially that as described by Lookingland et al. (31) and was recycled during use but made fresh each week. The output of the detector was to a Schimadzu Integrator. Peak heights were measured for each sample and referenced to the peak heights of various concentrations of the standards which were chromatographed randomly during the assay of the tissue samples.

DA and DOPAC were expressed per milligram of tissue protein for dam brain regions and per milligram of tissue wet weight for fetal brain. DOPAC:DA ratios were determined as an index of DA turnover. The data were initially analyzed with 3 (cocaine dose) × 3 (postinjection time) analyses of variance (ANOVAs).

Cocaine concentrations. Cocaine concentrations were determined in brain tissue of the mice from Exp-1 with a gaschromatographic-mass spectrometric procedure previously described (35,37) and are expressed as micrograms of cocaine per gram of tissue.

Histological Procedures

After observation for gross pathology, specimens from the dermis around the injection site, heart, liver, gastrointestinal (GI) tract, kidney, uterus, bladder and adrenal gland were dissected and fixed in 10% buffered formaldehyde. After dehydration, the tissues were embedded in paraffin, sectioned at 6 μm, mounted and stained with hemotoxylin and eosin. The histologies were read blind to route of cocaine administration.

TABLE 1

ORGAN PATHOLOGY (FREQUENCY) ASSOCIATED WITH COCAINE ADMINISTERED BY SUBCUTANEOUS INJECTION (SC) OR GAVAGE (G)

	Administration route	
Organ	SC (n = 5)	G (n = 6)
Mild to moderate		
Liver*	5	1
Moderate to severe	0	5
Kidney*	1	5
Skin*	4	0
Adrenal*	4	0

*Significant differences according to route of administration, $p \leq 0.05$, χ^2-test.

Statistical Procedures

The specifics of each analysis are provided in the Results section for each experiment. In general, the two groups in Exp-1 were compared with Student's t test, and frequency data were evaluated with chi-square tests. For Exp-2 and Exp-3, the primary analyses were ANOVAs, in some cases with repeated measures for time factors. Between-groups and repeated-measures factors are specified for the different dependent variables in the Results section with each experiment. In cases with significant interactions of the main variables, data were further analyzed with analyses of simple main effects followed by Duncan's test for multiple comparisons. In cases with heterogeneity of variance across treatment measures, data were natural-log-transformed prior to analysis.

RESULTS

Experiment 1. Effects of Cocaine on Female Mice: Influence of Route of Administration

Cocaine (60 mg/kg) given daily for 7 days reduced the body weights of female mice to about the same extent, regardless of the route of administration; weight loss (in grams; mean ± SEM): G: 10.3 ± 1.3 (11.6%), SC: 9.8 ± 1.3 (10.3%); $t(9) < 1$. Brain concentrations of cocaine at 30 min postinjection were ≈4 times greater when cocaine was administered by the SC than by the G route; cocaine (μg/g tissue; mean ± SEM): G: 4.22 ± 1.51, SC: 16.96 ± 1.49; $t(8) = 6.001$, $p < 0.001$.

Examination revealed no overt gross pathology of any organ and no histopathology of heart, GI tract, bladder or uterus for mice exposed to cocaine by either administration route or injected with saline. Histopathological examinations, however, distinguished G- and SC-injected cocaine mice from each other and from saline-injected mice. The distinguishing pathology according to cocaine administration routes is summarized in Table 1. The most common pathology associated with cocaine was hepatocellular degeneration and necrosis. This condition was rated as moderate to severe for most mice gavaged with cocaine, mild to moderate when the drug was SC injected and was not observed in the two saline-injected mice. Additional histopathological features that distinguished the two

routes of cocaine administration included kidney, dermis and adrenal pathology.

The histopathology that distinguished G animals from SC-injected mice included more severe liver damage (rated as moderate to severe) and interstitial nephritis of kidney that was recognized from aggregates of lymphocytes in the submucosal region of the renal medulla and pelvis. Criteria for severe liver pathology included: (a) larger numbers of altered hepatocytes, (b) the extension of lesions from the midzonal into the centrolobular region, (c) more severe hepatocellular degeneration and necrosis and (d) the deterioration of the architecture of the hepatic lobules and cords. The SC route of cocaine administration did not produce overtly observable dermal pathology (e.g., hair loss, open necrotic lesions, etc.). Histological analysis, however, revealed mild to moderate necrotizing dermatitis and myositis (moderate numbers of neutrophils and some macrophage) in samples taken from around the injection site and in hyperplastic cells in the subcapsular region of the adrenals (elongated fusiform-shaped cells in the zona glomerulosa and fasicularis). Neither of these conditions was observed in gavaged mice.

Experiment 2. Cocaine Effects on Pregnant Mice and Their Fetuses: Dose–Response Effects

Body weight and food consumption during pregnancy. Weight gains and food consumption during the time of cocaine treatment are summarized in Fig. 1. All mice gained weight during the treatment period (GD12–18); however, body weight during this time interacted with the type of treatment, $F(12, 246) = 18.455$, $p < 0.001$. As noted in the top panel of Fig. 1, body weights of the C40-injected mice became significantly reduced in comparison to SAL mice beginning on GD16 (i.e., after 4 daily injections of cocaine) and weights were significantly lower than both SAL and C20 mice on GD17 and GD18. Both the SAL- and the C20-treated mice had body weights 75% greater than their GD12 weight, whereas mice injected with the C40 dose gained significantly less weight during gestation (i.e., 55% of their GD12 weight), $F(2, 41) = 32.017$, $p < 0.001$. As noted in the bottom panel of Fig. 1, food consumption was also influenced by the cocaine treatments and the particular effect interacted with the number of daily injections, Treatment × Days $F(10, 205) = 2.440$, $p = 0.009$. An analysis of the simple main effects within days indicated that C20-injected mice had a transient reduction in food consumption that was significant only on GD14. In contrast, C40 mice had a significant reduction in food intake beginning with the first injection on GD12 (measurement taken the morning of GD13).

Motor activity after the final cocaine injection. The results of the activity tests are summarized in Fig. 2. These data were originally log-transformed to reduce heterogeneity of variance across the drug groups and were then subjected to a 3 (prenatal condition) × 3 (postinjection interval) × 3 (activity interval) ANOVA, with the last variable being a repeated measure. Of most importance for this analysis was a significant interaction of the three main factors, $F(8, 122) = 2.602$, $p < 0.01$. Resolution of this interaction with analysis of the simple main effects within each of the three postinjection times indicated that either dose of cocaine significantly elevated activity over that of vehicle-injected mice (≈11%) during the 30- and the 60-min postinjection tests. During the 120-min postinjection time assessment, the extent of the activity increase produced by cocaine was less than at earlier times but the increase was

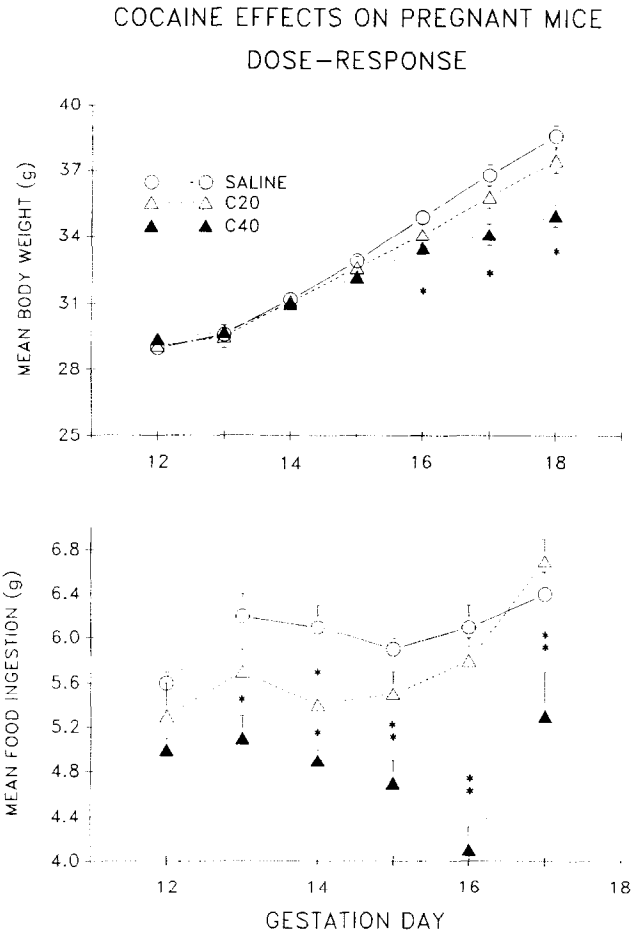

FIG. 1. Effects of daily SC injections of C20, C40 or SAL during GD12–18 on body weight (top) and food ingestion (bottom). Data points are mean ± SEM.

dose responsive with statistically significant increases of ≈4% and 8% for the C20 and C40 doses, respectively.

Litter size and fetal body and brain weights. In spite of the substantial reduction in weight gains of dams injected with the high dose of cocaine, litter size was unaltered by prenatal cocaine exposure (SAL: 8.1 ± 0.2; C20: 7.6 ± 0.3; C40: 7.6 ± 0.3). However, both body weight, $F(2, 41) = 2.431$, $p < 0.01$, and brain weight, $F(2, 41) = 3.851$, $p = 0.0293$, of GD18 fetuses were significantly reduced by maternal cocaine (Fig. 3). Post-hoc comparisons indicated that only the C40 dose produced statistically significant reductions in comparison with saline controls (12% and 6%, respectively) for body and brain weights.

Dopamine, DOPAC, and DOPAC:DA for pregnant mice and their fetuses. DA and DOPAC concentrations and DOPAC:DA ratios for pregnant mice at the different dose/postinjection interval combinations are summarized in Figs. 4 and 5 for the nucleus accumbens and the caudate, respectively. Cocaine did not alter DA concentrations in either nucleus at any dose or postinjection time.

DOPAC concentrations in the nucleus accumbens of the dam were reduced by cocaine; the particular effect depended on an interaction of dose and postinjection interval, $F(4, 52) = 3.74$, $p = 0.01$. Resolution of the interaction within each time

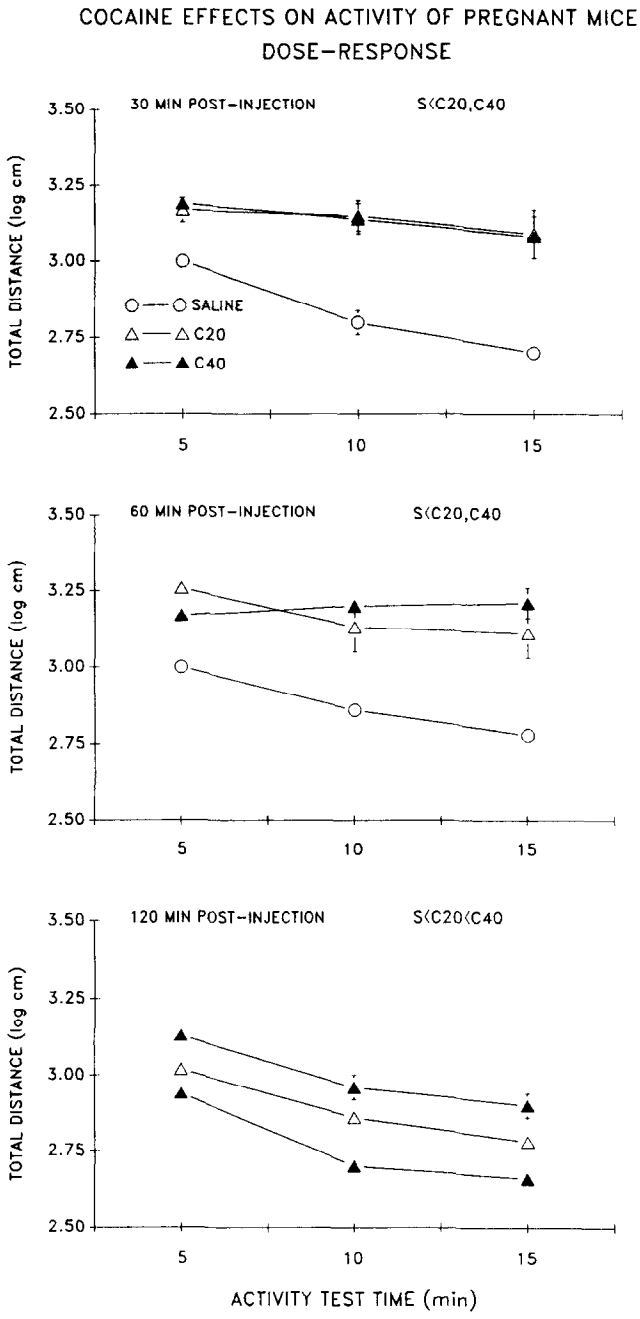

FIG. 2. Motor activity of pregnant mice (GD18) at 5-min intervals beginning 10, 40 or 100 min after the final of 7 daily injections of C20, C40, or SAL.

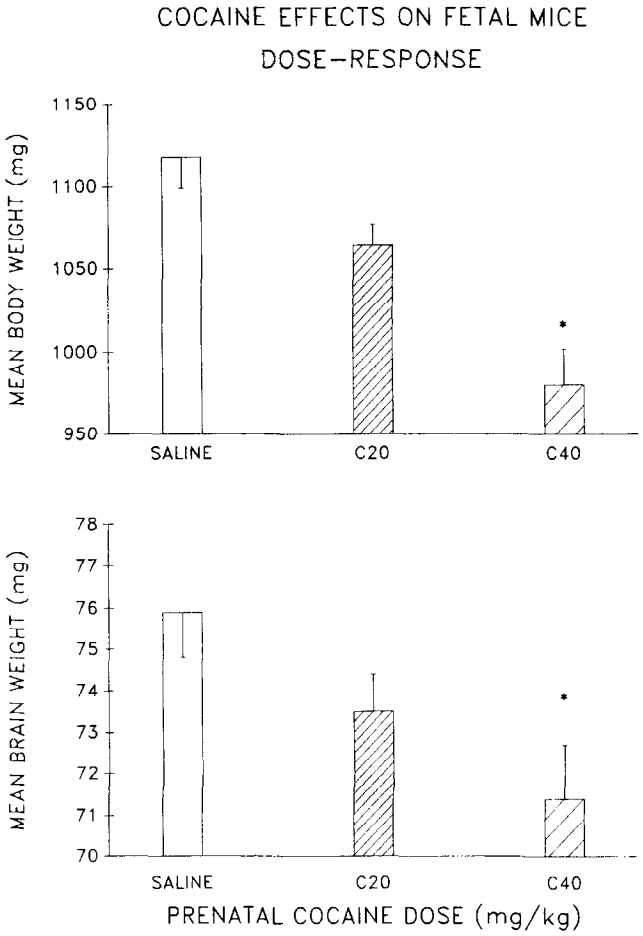

FIG. 3. Body weights (top) and brain weights (bottom) of GD18 fetuses of mice after 7 daily injections of SAL, C20, or C40.

interval indicated a systematic reduction in DOPAC with increasing cocaine dose at the 30-min postinjection time and no group differences at the later postinjection times. The apparent reduction in DOPAC for the C20 mice at 2 h postinjection is most likely a spuriously low measurement artifact. Of most importance are the noted changes in the DOPAC:DA ratios, which provide an estimate of DA turnover. The graphs suggest that cocaine attenuated the utilization of DA for at least 60 min postinjection. The ANOVA on these data indicated that the ratios varied according to an interaction of dose and postinjection interval, $F(4, 51) = 4.160$, $p = 0.005$. Resolution of the interaction indicated that DA utilization was significantly reduced by either dose of cocaine at 30- and 60-min postinjection periods. Although the ratio was significantly reduced for the C20 dose at 120 min, this reduction is most likely due to the previously noted aberrant low values of DOPAC for this group of mice.

DOPAC concentrations in samples obtained from caudate nucleus tissue (Fig. 5) were also reduced in cocaine-injected mice, $F(2, 56) = 2.904$, $p = 0.06$. The group means for cocaine-injected mice were lower at all time points and were systematically related to dose at the 30-min point; however, the Dose × Time interaction for these data were not significant. The DOPAC:DA ratios were also reduced in cocaine-injected mice, and this effect interacted with time, Dose × Time $F(4, 51) = 2.500$, $p < 0.05$. Resolution of the interaction indicated that the DOPAC:DA ratio in this tissue was reduced by cocaine only at the 30-min time point, $F(2, 51) = 3.768$, $p = 0.030$.

In contrast to its effects on DA systems of pregnant mice, cocaine did not alter DA or DOPAC concentrations or their ratios in the caudate nucleus of their fetuses. Caudate of SAL control fetuses had DA concentrations of approximately 0.664 ng/mg tissue and DOPAC concentrations of approximately 0.104 ng/mg tissue. These concentrations were approximately

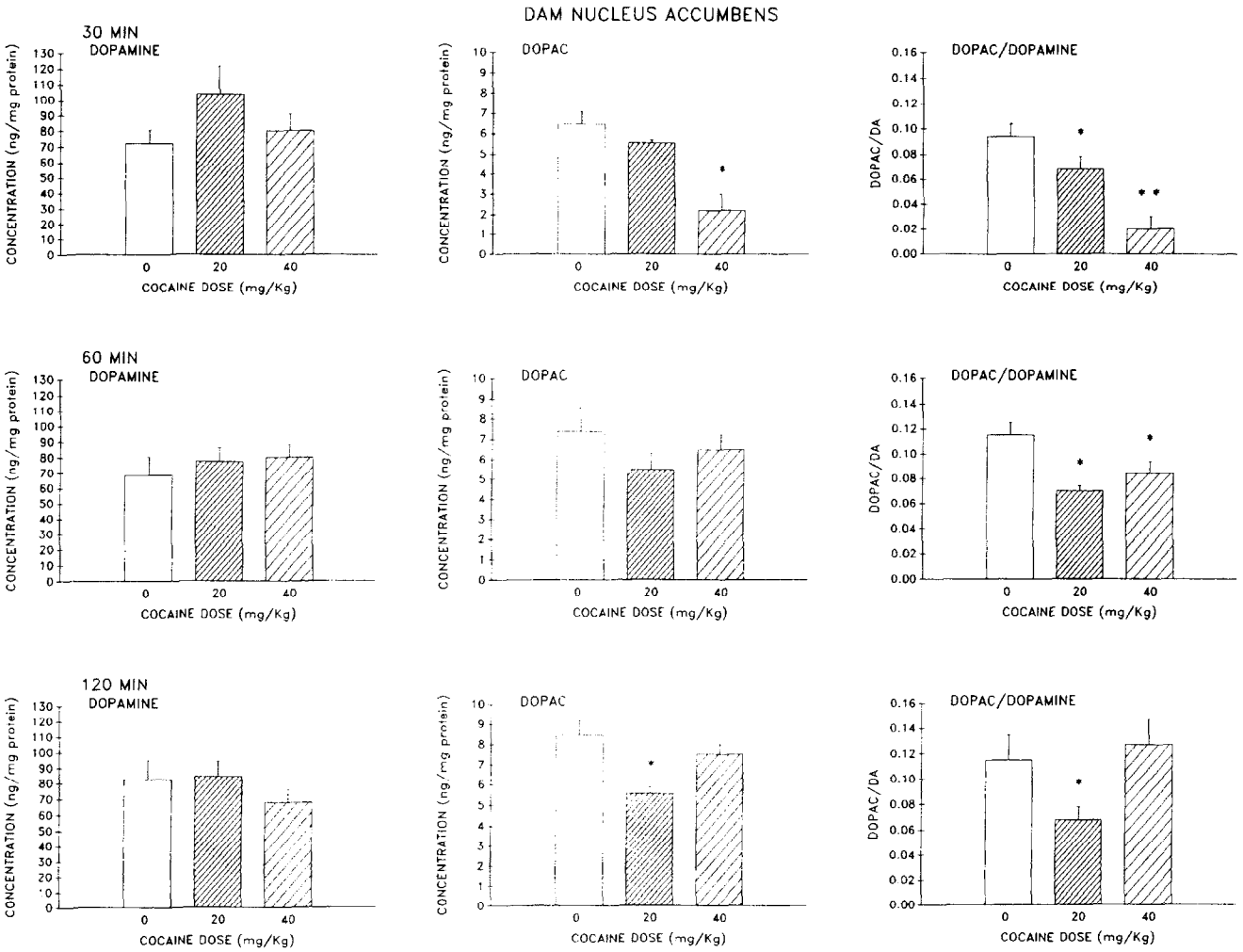

FIG. 4. DA and DOPAC concentrations and DOPAC:DA ratios in nucleus accumbens of pregnant mice at 30, 60 and 120 min after the final of 7 daily injections of SAL, C20 or C40.

2% and 3% of comparable DA and DOPAC values for the caudate of the SAL dams.

Experiment 3. Cocaine Effects on Pregnant Mice and Their Fetuses: The Role of Undernutrition

The results of this experiment are summarized in Fig. 6. As for Exp-2, the body weight of pregnant mice (Fig. 6, upper panel) during the course of the 7 daily injections depended on the treatment condition: Treatment Condition × Time $F(12, 132) = 6.301$, $p < 0.001$. Resolution of the interaction indicated that body weights of C40-injected mice did not differ from SPF mice at any point in the treatment, that C40-injected mice weighed significantly less than SAL mice beginning on GD14 as was noted in Exp-2, and that SPF mice weighed significantly less than SAL mice beginning on GD15. In addition, as noted in Exp-2, fetal body weight, $F(2, 22) = 3.188$, $p = 0.06$, and brain weight, $F(2, 22) = 5.845$, $p < 0.01$, were significantly reduced by the C40 dose of cocaine. In contrast, in spite of reducing the dams' body weight gain to the about the same extent as for those injected with cocaine, the pair-feeding procedure had no effect on body or brain weights of the GD18 SPF fetuses.

DISCUSSION

The results of the three experiments help characterize the effects of cocaine on pregnant C57 mice and form the basis of a murine model to study the effects of maternal cocaine exposure on offspring. Knowing this information will facilitate the comparison of results obtained from using this model in future experiments to studies using other cocaine-exposure conditions and will establish the generalization of our results to other species including humans.

Cocaine Effects According to Administration Route

The organ pathology and the drug concentrations produced in brain by the high dose of cocaine (60 mg/kg) depended on whether the drug was gavaged or injected SC. Animals gavaged with cocaine, although having relatively low brain concentrations of the drug (4.2 μg/g), exhibited moderate to severe damage to the liver and interstitial nephritis of the kidney. The observed liver damage is consistent with previous reports for mice administered cocaine (44,47), including the C57 strain used in the present study (41). Possible mechanisms for this effect and similar pathology in humans were discussed by Shuster et al. (47). The relative greater effect of cocaine on

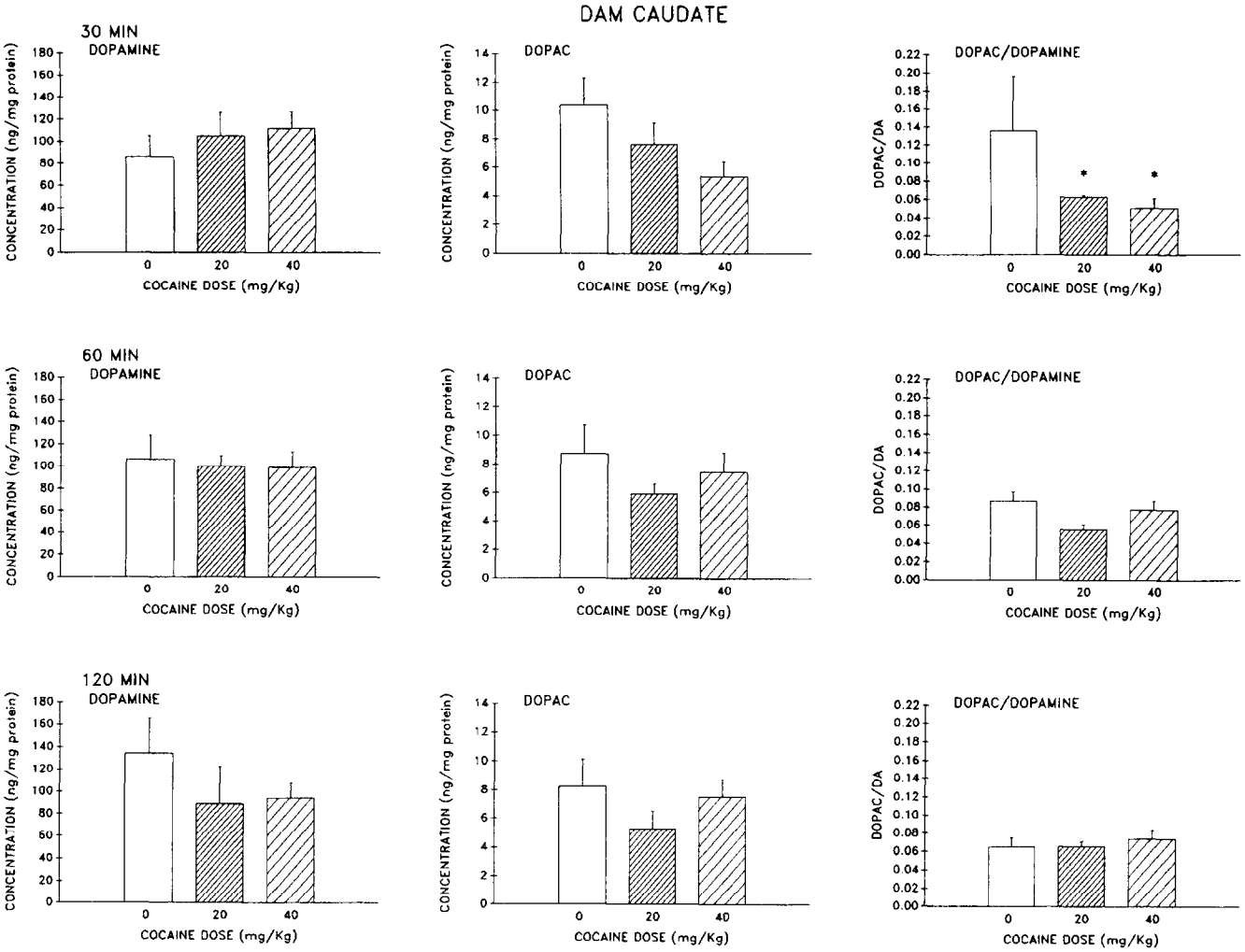

FIG. 5. DA and DOPAC concentrations and DOPAC:DA ratios in caudate nucleus of pregnant mice at 30, 60 and 120 min after the final of 7 daily injections of SAL, C20 or C40.

liver when gavaged rather than SC injected is not unexpected because of the high concentration of cocaine delivered directly to the liver from the GI tract. The nephritis noted in animals gavaged with cocaine was not observed in SC-injected mice. To our knowledge, interstitial nephritis associated with cocaine use has not been previously reported in the literature and its mechanism is not readily apparent.

SC injections of cocaine produced mild necrotizing dermatitis and myositis surrounding the injection site, mild liver damage and some slight cellular changes in the subcapsular region of the adrenals. The dermatitis associated with SC cocaine injections in this study was very mild in comparison with previous reports of their effects on rats (e.g., hair loss and ulcerated pustular necrotic lesions of the epidermis) (3,14). In the present study, there was no hair loss or other overt manifestations of the injections; effects were revealed only during histological examination. The mild dermal effects of SC cocaine observed in our study relative to the earlier reports may be related to species (mouse vs. rat), pigmentation (pigmented vs. albino) or possibly to injection technique. Injection techniques is suggested because of our observation that leakage of cocaine from the SC space to the epidermal surface can produce substantial necrosis in the C57 mouse. This condition can be avoided by evacuating the syringes well beyond the site of needle entry. The mild adrenal pathology observed for SC-injected mice is of unknown cause but has been observed in aged mice (15).

The fourfold higher brain concentration of cocaine for SC-injected mice vs. that of G mice was not unexpected because gavaged cocaine is subject to presystemic metabolism prior to distribution to the brain. The substantially higher concentration for the SC-injected vs. G mice in this experiment is consistent with unpublished values obtained from plasma of C57 mice collected 15, 30, 45 and 60 min after G or SC injections (K. Patrick, Medical University of South Carolina).

The overall results of Exp-1 provide no dominating logic for selecting one route over the other for cocaine administration. Thus, because SC injections produced substantially higher brain concentrations without an increase in weight loss or organ pathology, this route was selected for cocaine administration in Exp-2 and Exp-3.

Cocaine Effects on Pregnant Mice and Their Fetuses

C40 given daily during GD12–18 in Exp-2 reduced food ingestion and weight gain. In addition, the body and brain weights of the GD18 fetuses of these dams were reduced

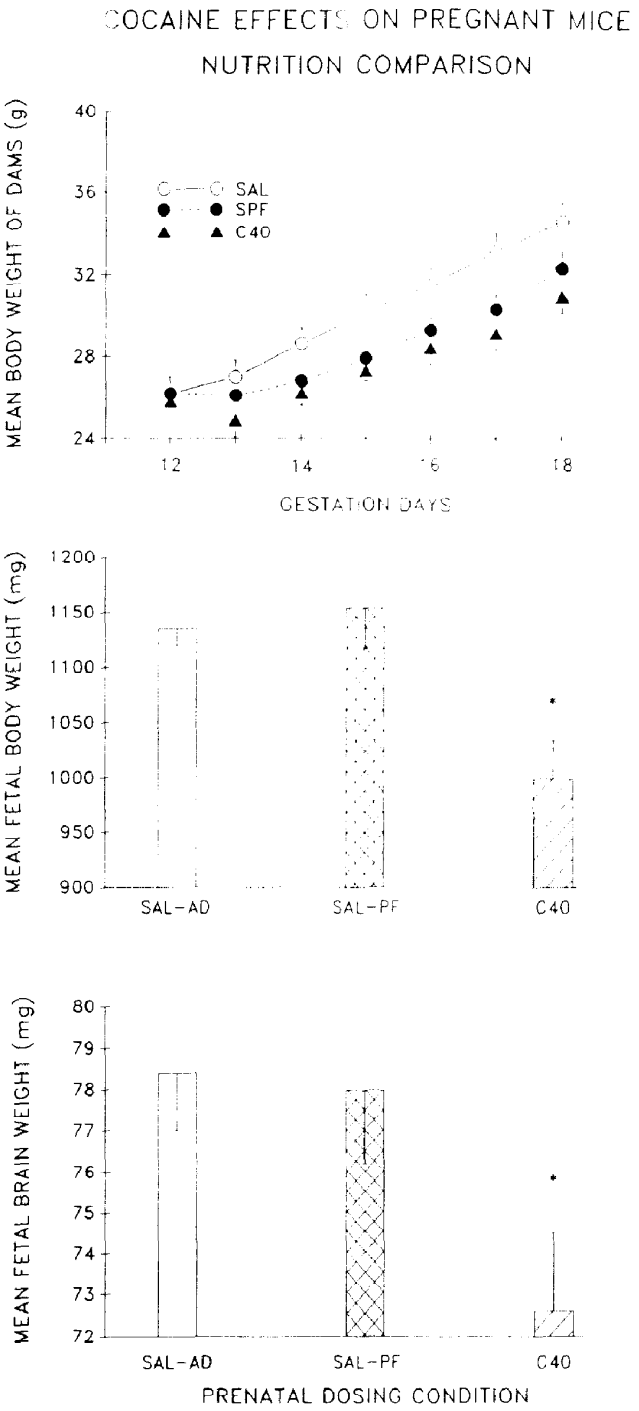

FIG. 6. Effects of daily SC injections of C40 or saline (SAL, SPF) during GD12–18 on body weight (top). SAL mice had food available ad lib. SPF mice were given amounts of food comparable to C40 mice. Middle and bottom graphs reflect body weights (middle) and brain weights (bottom) of GD18 fetuses for the three groups.

in comparison with their non-food-deprived saline controls. Because the C20 dose did not produce comparable effects, the threshold for these perinatal effects in C57 mice must be between 20 and 40 mg/kg. The results of Exp-3 essentially duplicated the effects of the C40 dose on weight gain of the dams during GD12–18 and on the body and brain weights of GD18 fetuses. Maternal undernutrition alone cannot account for the reductions in fetal body and brain weight because such reductions were not observed for the fetuses of pair-fed dams (SPF) who exhibited attenuated weight gains during pregnancy similar to that of cocaine-injected dams. The absence of fetal body and brain weight reductions for the food-deprived mice is not surprising because a number of reports have indicated that offspring development, particularly the brain, is frequently maintained at the expense of the dam under conditions of substantial undernutrition (12). Although maternal undernutrition appears an unlikely mediator of the reduced body and brain weights of the cocaine-exposed fetus, fetal nutritional insufficiency resulting from a drug-induced reduction in placental transfer or other mechanisms remains a possibility. The somewhat greater extent of the reduction in body than in brain weights for fetuses exposed to the high cocaine dose (i.e., 12% vs. 6%) suggests that the brain may have been somewhat spared from the detrimental effects of maternal cocaine. Substantial reductions in fetal body weights have been reported in the absence of brain weight reductions under conditions of maternal undernutrition and preserving brain growth is the typical pattern for developing animals (12). However, although the extent of the brain weight reduction was somewhat less than that for overall body weight in our experiments, it occurred only when there was also a body weight reduction (i.e., not for the C20 or the SPF groups). In addition, the brain:body weight ratio was only slightly altered for the C40 fetuses (1:14) in comparison with SAL fetuses (1:14.5), suggesting a symmetrical weight reduction with little sparing of the brain.

Although our primary interest is to study the long-term postnatal effects of prenatal cocaine exposure, the present study helps to establish the comparability of our murine model to the reported effects of cocaine on humans and other rodent models. The prenatal effects of cocaine obtained with our murine model and similar effects reported for humans are summarized in Table 2. In comparing these effects, the higher dose of cocaine used in our experiments (40 mg/kg) produced effects on the fetus quite comparable to those reported for neonates of cocaine-using women. For example, the body weight reductions of 12% noted in our two experiments closely match those reported by Chasnoff (5,8) and by Zuckerman et al. (52) for babies (Table 2). Furthermore, the 6–7% reduction in brain weight is not far off the percentage of reduction in head circumference reported for cocaine babies. The somewhat greater percentages of reduction in brain size noted in our mouse studies may merely reflect differences in methods of estimating brain size (i.e., weight vs. head circumference) rather than a biological difference.

It has been difficult to rule out the contribution of other drugs and undernutrition to the observed reduction in birth weights of cocaine-exposed babies (43); however, our experiments and other reports using rodent models (9) indicate that cocaine in the absence of other drugs can reduce birth weight. Furthermore, the results of the present study also indicate that the maternal undernutrition accompanying cocaine exposure cannot alone account for the fetal body and brain weight reductions. Thus, factors other than maternal undernutrition could account for the low birth weight of cocaine-exposed babies. The present study suggests that the amount of cocaine to which the fetus is exposed might be a critical factor in distinguishing the studies that note detrimental effects from those that do not. The lower dose of cocaine used in our experiments (20 mg/kg), although stimulating pregnant mice to about the same degree as the higher dose, had very minor and nonsignificant effects on the perinatal measures. Thus,

TABLE 2

EFFECTS (%) OF MATERNAL COCAINE ON PREGNANCY CHARACTERISTICS IN HUMANS AND MICE

	C57 mice		Humans	
Effect	Exp-2	Exp-3	Chasnoff et al. (7)	Zuckerman et al. (51)
Reduced weight gain for dam	21	18	5.2	25
Reduced birth weight	12	12	17	12
Reduced brain size (weight, circumference)	5.5	7.4	5.5	3.8

maternal cocaine in C57 mice, depending on the particular dose, can produce effects consistent with the contrasting reports for human cocaine users (26). The presence or absence of the effects of maternal cocaine on the newborn in the human literature might be accounted for by the amount of cocaine exposure, a condition not readily testable in humans, but easily addressed with our murine model, a model that has several features common to maternal cocaine exposure in humans.

An important issue to be considered when generalizing from an animal model to humans is dosing. Cocaine doses of 20 and 40 mg/kg in the present experiments produced 30-min plasma concentrations near 1.3 and 2.8 μg/ml (35). Although the plasma concentration produced by the higher cocaine dose might appear to be unrealistic from the human use perspective, the dynamics of the drug in the present study suggest that the C40 dose is not unacceptably high for C57 mice. For example, this dose did not increase mortality in either the dam or the fetus, nor did it produce stereotypic behavior that normally occurs with high doses of cocaine during activity tests, at least to the extent that it attenuated elevated locomotion. In addition, the maximum stimulatory dose of cocaine for C57 mice is near 32 mg/kg (46) (i.e., between the C20 and C40 doses used in our experiments). Finally, both doses altered DA turnover (DOPAC:DA) in nuclei accumbens and caudatus. The reduction in DOPAC concentrations observed in this experiment were comparable to those reported for male ICR mice following injections of cocaine (21). The effects on the nucleus accumbens are particularly important because DA in this nucleus has demonstrated importance in the reinforcing effects of cocaine (25,29). Thus, based on these dynamics, the doses of cocaine selected for our experiments appear to be reasonable approximations of levels to which humans might be exposed.

Our purpose of assessing concentrations of DA and its metabolite in the GD18 fetal brain was to determine if the effects of cocaine on fetal DA systems were similar to its effects on the adult brain. Such evidence might in turn suggest possible mechanisms for the long-term effects of maternal cocaine on offspring DA systems and the behaviors they mediate (4,22–24,28,36,38). Experiments by Meyer et al. (33) have established cocaine binding sites, and maternal cocaine administration can stimulate tyrosine hydroxylase activity in the fetal brain (32). Unfortunately, the present study provides no evidence for altered DA turnover, which would be expected with altered activity of its regulatory enzyme. Future studies evaluating the nucleus accumbens and caudate may provide such evidence because in adult animals at least the two areas are reported to respond differently to cocaine (18). This difference is also apparent in the adult pregnant mice of the present study because the effects of cocaine were more restricted for the caudate than for the nucleus accumbens. Regarding the absence of effects on DA systems of the fetus, the peak brain concentrations of cocaine obtained in our experiment were approximately sevenfold higher in the dam's brain than that in her fetuses, although cocaine was present in the fetuses for a more prolonged period (35). Presumably, as was noted for ethanol (1,39,40), the peak value rather than duration of exposure is the more relevant parameter in producing effects on the fetal brain. Clearly, however, maternal cocaine exposure at doses that altered DA turnover in both the nucleus accumbens and caudate of her brain did not alter DA turnover in caudate of her fetuses.

In summary, the present study indicates that the skin ulceration and necrosis reported following SC injections of cocaine in rats are not observed in C57 mice and that the SC route yields higher brain concentrations than gavage, without an increase in peripheral pathology. At doses that produce plasma concentrations consistent with its stimulatory effects, cocaine produced reductions in food ingestion and weight gains during pregnancy and reductions in fetal body and brain weights at term. The extent of the reductions are quite comparable to reports on babies prenatally exposed to cocaine. The present study also suggests that maternal undernutrition is not a likely mediator of these perinatal effects and that differences in amount of cocaine exposure might be a likely candidate for the contrasting effects of maternal cocaine in the human literature.

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