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Comparison of Oral and Subcutaneous Routes of Cocaine Administration on Behavior, Plasma Drug Concentration and Toxicity in Female Rats

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DOW-EDWARDS, D., T. A. FICO, M. OSMAN, Z. GAMAGARIS AND D. E. HUTCHINGS. *Comparison of oral and subcutaneous routes of cocaine administration on behavior, plasma drug concentration and toxicity in female rats.* PHARMACOL BIOCHEM BEHAV **33**(1) 167–173, 1989.—Oral and subcutaneous routes of administration of cocaine HCl were investigated in female Wistar rats for food and water consumption, locomotor activity, stereotypic behaviors, plasma drug concentrations and injection site pathology. Animals received either 40 or 80 mg/kg/day by gastric intubation (PO-40 and PO-80 respectively) or 20 or 40 mg/kg/day subcutaneously (SC-20 and SC-40). All groups received the drug or the vehicle for 16 consecutive days. Locomotor activity and stereotypy were evaluated on Days 1, 5, 10, and 15. Plasma drug concentrations and injection site pathology were determined on Day 16. Subcutaneous administration was associated with a sensitization to the effects of cocaine on locomotion and stereotypy, higher blood levels than oral administration at the same dose, and severe dermal lesions. However, there were no differences in any measure between the SC-20 and SC-40 groups. Oral cocaine was also associated with behavioral sensitization. However, unlike the SC route, oral cocaine was characterized by dose-related increases in locomotion and stereotypy in the absence of gastrointestinal pathology. Inasmuch as oral administration resulted in dose-response relationships and low toxicity while subcutaneous administration did not, these factors should be considered in future studies utilizing chronic cocaine administration.

Stereotypy Locomotor activity Plasma concentrations Oral Subcutaneous Food and water consumption Cocaine

RECENT reports of the reproductive and developmental toxicity of prenatal cocaine exposure [e.g., (3)] have led to an effort to develop an appropriate animal model. An unresolved issue has been the selection of a route of chronic administration that allows pharmacologically relevant doses to enter the fetus while minimizing maternal toxicity. Although intranasal administration is a preferred route among cocaine users, it is not appropriate for rodents. Unlike humans, venous drainage from the nasal mucosa in the rat does not have access to the circular sinus and pituitary so that compounds administered intranasally in the rat would be expected to have different pharmacodynamics than following intranasal administration in man. Intravenous (IV) and intraperitoneal (IP) administration would probably result in rapid uptake and distribution but each poses serious methodological problems: The IV route would necessitate maintaining indwelling catheters and is not a practical technique for studies requiring a large sample size. A problem with IP administration is that extraplacental transfer of the drug can occur such that the compound is delivered both directly across the uterine wall as well as through the maternal circulation. The remaining alternatives are oral (PO) and subcutaneous (SC) routes.

The SC route has been used to administer cocaine to rodents but at least one study has reported the occurrence of necrotic lesions at the injection sites (2). We have been unable to find similar studies examining the oral route but gastric lesions may be produced and hepatic metabolism following first pass though the liver may reduce plasma levels substantially. We thus compared the SC and PO routes of administration for locomotion, stereotypy, plasma cocaine concentrations and associated pathology at the injection site.

METHOD

Animals

Upon their arrival, female Wistar rats, 175–200 g (Hilltop Lab

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Animals, Inc., Scottdale, PA), were housed in groups of three in Plexiglas cages on wood chips for one week. During this acclimation period food and water were available ad lib.

Drug Administration

On the first day of the study, the rats were randomly assigned to a mode of administration group (SC or PO) and placed in individual cages constructed of clear polypropylene and measuring 45 × 23.5 × 20 cm. The animals in the SC group received 20 or 40 mg/kg cocaine HCl in a volume that corresponded to 1 ml/kg body weight (2 and 4% solutions). The animals in the PO group received 40 or 80 mg/kg cocaine HCl in a volume ranging from 1.5 to 2.5 ml (0.4 and 0.8% solutions). For both routes the drug was dissolved in sterile water and prepared freshly for each of the 16 consecutive days of administration. Controls received the vehicle equivolumetric for each mode of administration. Food and water intake for each rat were recorded each day between 10.00 and 11.00 hours from the first day of treatment until Day 15. Food intake was determined by placing food pellets in stainless steel hanging food dispensers and weighing the dispenser each day. Water was offered in 240-ml glass bottles and the amount consumed was determined by weighing the container each day. Food (in g) and water (in ml) consumptions were divided by the daily body weights to allow for statistical comparisons between groups.

Behavioral Testing

On Days 1, 5, 10 and 15, locomotor activity and stereotyped behavior were monitored for 3 hr immediately following the administration of the drug or vehicle. The animals were placed in cages similar to their home cages and both were placed on a 5-channel electronic activity monitor with 5 remote sensors (Stoelting Co., Chicago, IL). The five sensors, driven by a single oscillator, were cross-calibrated allowing comparison of data between sensors. The 5 sensors were stacked in a vertical metal rack with metal shelving acting as an insulator between the respective sensor fields. Testing revealed no "cross-talk" (i.e., heterodyning) between sensors. For the present study, the threshold reset time was placed in the normal mode and the activity level and mA controls set at 15 and 0.7 respectively. Activity counts were collected at 60-sec intervals for three hours using an IBM XT computer interface. Neither food nor water was available during testing.

Stereotypy was scored by an investigator, unaware of the treatments, in 15-sec observation periods at 20-min intervals for the three hours during which the activity levels were also recorded. The cages were situated such that the observer could see each animal from outside the room where the recordings were made. Stereotyped behavior was assessed by scoring for the presence or absence of the following: sniffing; repetitive head or limb movement; grooming; biting, chewing or gnawing of bedding or forepaws; backward locomotion and lateral head swaying. The presence of a given behavior was a score of one allowing a maximum score of 6 at each of the 9 periods of observation and a maximum possible score of 54 for each animal for each test day. The total score for each animal was then used to compute the group mean which was subsequently used for statistical comparisons.

Plasma Cocaine Concentrations

On Day 16, the rats were sacrificed by decapitation 15 or 45 min after drug administration. Trunk blood was collected in chilled glass Vacutainers and centrifuged at 4°C. Plasma was decanted into polypropylene vials to which NaF had been added (final concentration 0.5% NaF). Samples were stored in a −70°C freezer until they were packed in dry ice and sent to the Laboratory for Human Toxicology at the University of Utah where GC/MS analysis was performed.

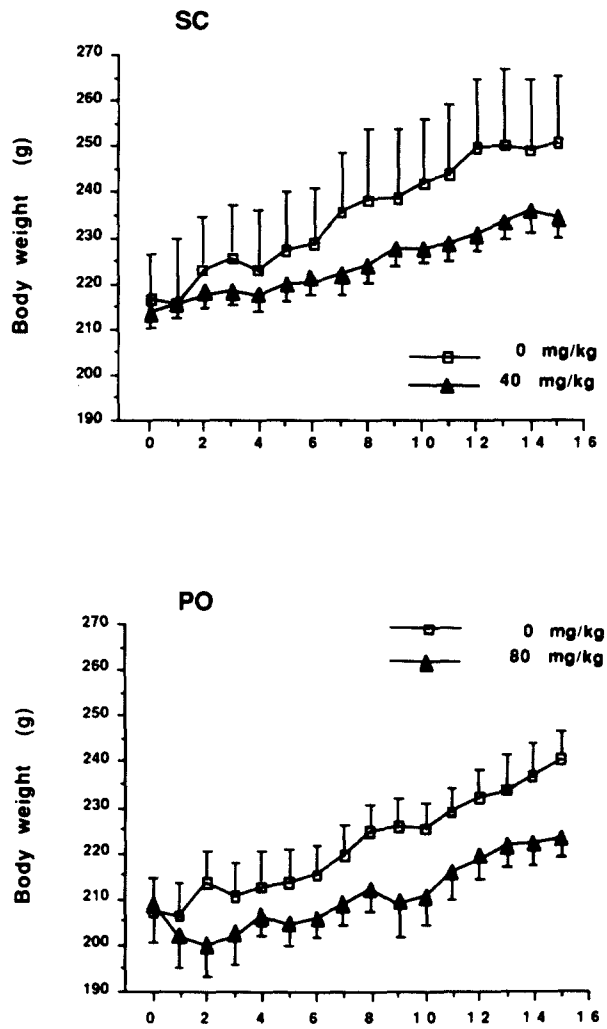

FIG. 1. Body weights (mean ± sem) of the high dose and control animals in the SC (top) and PO (bottom) treatment groups over 15 days of the study (Day 0 is the initial or pretreatment body weight). N=4 for the control groups and 7 for the treated groups. Total weight gain was significantly decreased by the PO-80 treatment compared to the PO-0 control (univariate F-test, $p<0.05$).

Necropsy

Necropsies were performed on all animals by a pathologist unaware of the route of administration and dose utilized. Tissue from the esophagus, stomach, duodenum, and dermis was removed for histopathological analysis. Tissue specimens were fixed with 10% buffered formalin, embedded in paraffin, sectioned at 6 μm and stained with hematoxylin and eosin (H&E).

Statistical Analyses

Results were analyzed using the SYSTAT statistical program

on an IBM PC. ANOVA or repeated measures ANOVA was followed by a univariate F-test or Student's *t*-test where appropriate. Nonparametric data were analyzed by Kruskal-Wallis ANOVA followed by Mann-Whitney U-tests where appropriate

RESULTS

Toxicity and Mortality

Of the 32 animals receiving cocaine in this study, lethal cocaine-induced seizures were observed in two animals: one PO-40 animal on Day 7 and one SC-40 animal on Day 15. Although six animals were observed to show ataxia followed by a nonlethal seizure, only one animal showed such an effect during behavioral testing. Of these, four were in the PO-80 group, one each in the SC-40 and the PO-40 groups and all occurred between 13 and 16 days of treatment.

Body Weight, Food and Water Consumption

Figure 1 shows mean body weights over 15 observation days in the high dose and control SC and PO groups with Day 0 being the initial preinjection body weights. Cocaine administered by either route inhibited weight gain, although only the PO groups showed a dose-related decrease, $F(2,15)=5.22$, $p=0.02$. (The animal which died was not included in the analysis.) The PO-80 group actually lost weight over the first three days of treatment. Thereafter, the rate of weight gain approached that of the control group.

Food consumptions for the SC and PO control and high dose groups are shown in Fig. 2. The upper panel shows a moderate reduction in food consumption among the SC-40 animals on the first 6 days following the initiation of drug treatment. Subsequently, tolerance to the anorexic effects of the drug developed. In comparison, food consumption of the PO-80 animals (lower panel) was lower than that of the PO-0 group for only the first two days of treatment.

Water consumption in ml per kg body weight is shown in Fig. 3. The upper panel shows that compared with the SC-0 group, the SC-40 animals consumed more water during the last 8 days of treatment. A one-way repeated measure analysis of variance (ANOVA) revealed a significant effect for treatment, $F(2,17)=4.08$, $p=0.036$ (the animal which died on day 15 was included in this analysis) and a univariate F-test showed that the high dose group differed reliably from the SC-0 and SC-20 groups. For the PO groups, there were no statistically significant effects of treatment on water consumption.

Locomotor Activity

Mean total activity levels for the four test days are shown for all groups in Fig. 4. Activity levels for both the SC-0 and PO-0 control groups remained relatively constant throughout testing. By comparison, activity not only increased among all of the cocaine-treated groups, but also tended to increase further as testing continued.

Activity levels of the SC groups are shown in the upper panel of Fig. 4. On Day 1 of testing, there were no statistically significant differences in the activity levels of the three groups, however the SC-40 group was 3-fold more active than the control group. On Day 5, the activity of both cocaine groups increased substantially, peaked at Day 10, and appeared to be asymptotic at Day 15. A one-way repeated measures ANOVA yielded a significant effect for both treatment, $F(2,16)=22.49$, $p=0.001$, and test day, $F(3,48)=17.97$, $p=0.001$. The post hoc analysis revealed that there was no difference between the SC-20 and SC-40

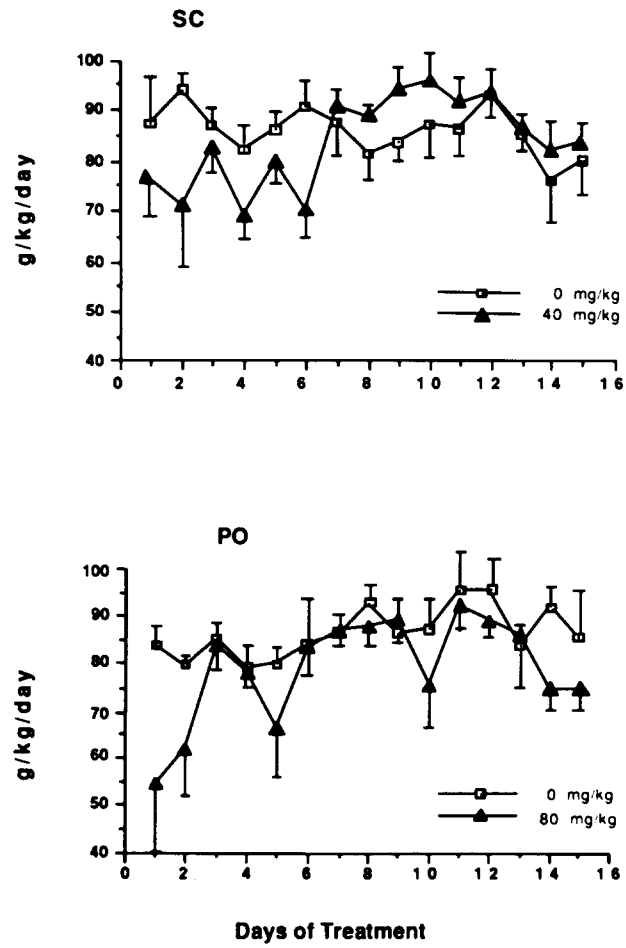

FIG. 2. Food consumed (g/kg body weight/day) in control and high dose cocaine-treated animals during 15 consecutive days. Each point represents the mean ± sem of either 4 control animals or 7 cocaine-treated animals.

groups on any test day but that both were significantly more active than the control group.

The PO cocaine groups (shown in the lower panel, Fig. 4) also demonstrated an overall increase in activity with repeated cocaine injections. A one-way repeated measures ANOVA revealed a significant effect of treatment, $F(2,12)=11.26$, $p=0.002$. After Day 1, activity of the PO-40 group increased somewhat, and then remained relatively stable. By comparison, the PO-80 group exhibited activity levels that were substantially higher than the other 2 groups; post hoc analysis revealed that the PO-80 group was more active than the controls on Days 1, 10, and 15 and more active than the PO-40 group on Day 10. Inasmuch as the activity levels of both the PO-0 and PO-40 groups did not change significantly over testing, ANOVA did not yield a statistically significant effect for test day.

Stereotypy

Mean total stereotypy scores for the four test days are shown for all groups in Fig. 5. Overall, the pattern of stereotypy scores for both treated and control groups was nearly identical to the pattern of activity levels. Stereotypy scores for both the SC-0 and PO-0 control groups were relatively stable throughout testing, whereas all cocaine-treated groups showed increased scores,

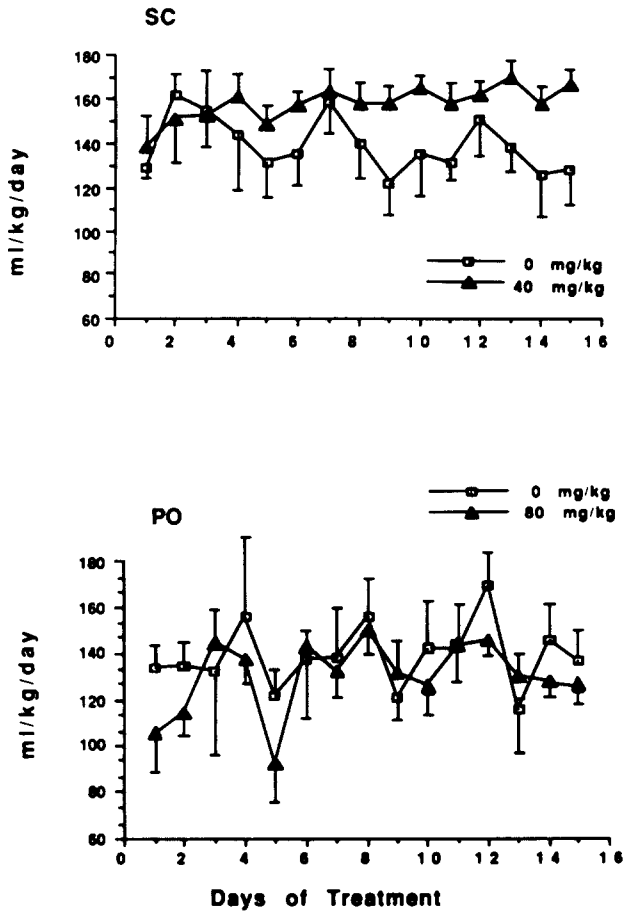

FIG. 3. Water consumed (ml/kg body weight/day) in control and high dose cocaine-treated animals during 15 consecutive days. Each point represents the mean ± sem of 4 control animals or 7 cocaine-treated animals.

particularly after Day 1.

As shown in the upper panel of Fig. 5, stereotypy scores for both SC cocaine groups were somewhat elevated on Day 1, increased substantially on Day 5, and remained asymptotic for the remainder of testing. The Kruskal-Wallis ANOVA revealed a significant effect of treatment on Day 5, $\chi^2(2)=8.32$, $p=0.016$, Day 10, $\chi^2(2)=9.04$, $p=0.011$, and Day 15, $\chi^2(2)=9.29$, $p=0.01$, but no difference between the cocaine-treated groups. As shown in the lower panel of Fig. 5, scores for both PO cocaine groups were somewhat elevated on Day 1. The scores of the PO-40 group increased on Days 5 and 10 and increased still further on Day 15. The Kruskal-Wallis ANOVA showed that both cocaine groups had significantly greater scores on Days 5, $\chi^2(2)=10.83$, $p=0.004$, 10, $\chi^2(2)=7.78$, $p=0.02$, and 15, $\chi^2(2)=7.13$, $p=0.028$. In addition, on Days 5 and 10 the PO-80 group had significantly greater stereotypy scores than the PO-40 group.

Plasma Cocaine Concentrations

Figure 6 shows mean plasma cocaine levels 15 and 45 minutes after SC and PO administration on Day 16. The upper panel of Fig. 6 shows that between 15 and 45 minutes, the mean plasma concentration of cocaine doubled for the SC-20 group. The mean concentration for the SC-40 group was approximately 2 times that of the SC-20 group at 15 minutes but at 45 minutes was not

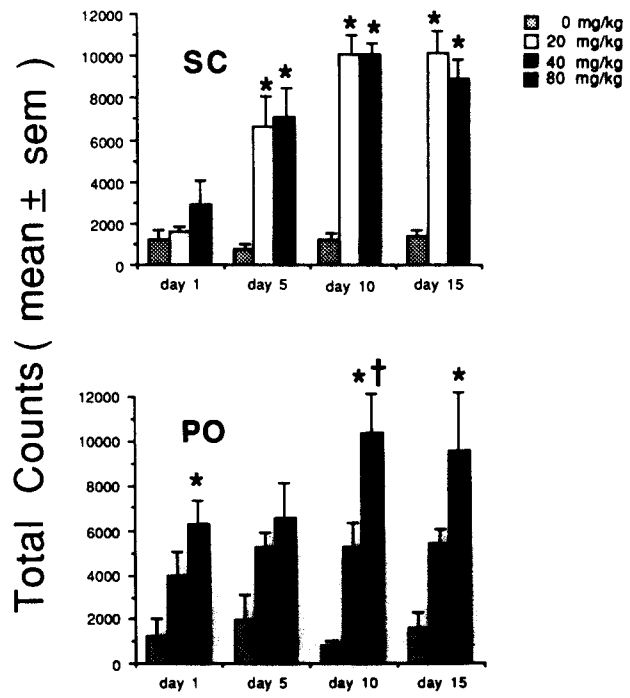

FIG. 4. Total activity counts for PO and SC cocaine administration collected for 3 hours immediately following the dose of cocaine. N=4 for the control groups and 7 or 8 for the treated groups. *$p<0.05$, significantly different than vehicle-injected control and †$p<0.05$, significantly different from PO-40 group, univariate F-test.

different. ANOVA revealed a significant effect for dose, $F(1,11)=6.47$, $p=0.03$, but not for time.

The lower panel shows that between the sample periods, the mean concentrations for both the PO-40 and PO-80 groups declined by about 50 percent. The mean concentration for the PO-80 groups was higher than that of the PO-40 group at both sample times. ANOVA revealed a significant effect for dose, $F(1,10)=13.47$, $p=0.004$, but not time.

Necropsy

Visual inspection of the dermis from the SC cocaine groups showed severe and extensive lesions at the injection sites. These were associated with epidermal ulceration and subcorneal pustules in the necrotic epidermis. Although the SC controls did show some abscesses, these were far fewer and considerably smaller than in the SC cocaine groups.

Microscopic examination of SC cocaine groups showed severe inflammatory reactions that were both acute and chronic. These were manifested by areas of necrosis, loss of cellular details and an increase in the number of leukocyte and monocyte infiltrates in the sites of injection as shown in Fig. 7a. A much lower number of cellular infiltrates was observed in the histopathologic examination of the SC controls (Fig. 7b).

Among the PO animals, neither gross nor microscopic examination of esophageal, stomach or duodenal tissues revealed any lesions, nor did the animals show any obvious dermal abnormalities. Of the SC-injected animals examined, none showed any esophageal, stomach or intestinal lesions.

DISCUSSION

Comparison of oral and subcutaneous routes of cocaine admin-

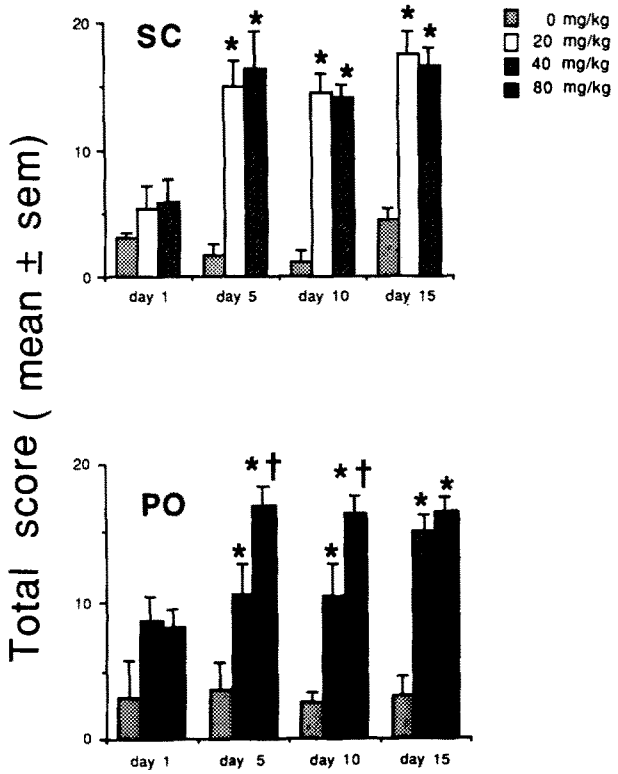

FIG. 5. Total stereotypy score (group mean ± sem) following PO and SC cocaine administration. Animals were scored by observers unaware of the experimental treatments for 15-second intervals every 20 min for a 3-hour observation period. Maximum possible score on each test day is 54 for each animal. *$p<0.05$, significantly different from vehicle-injected control and †$p<0.05$, significantly different than PO-40 group, univariate F-test.

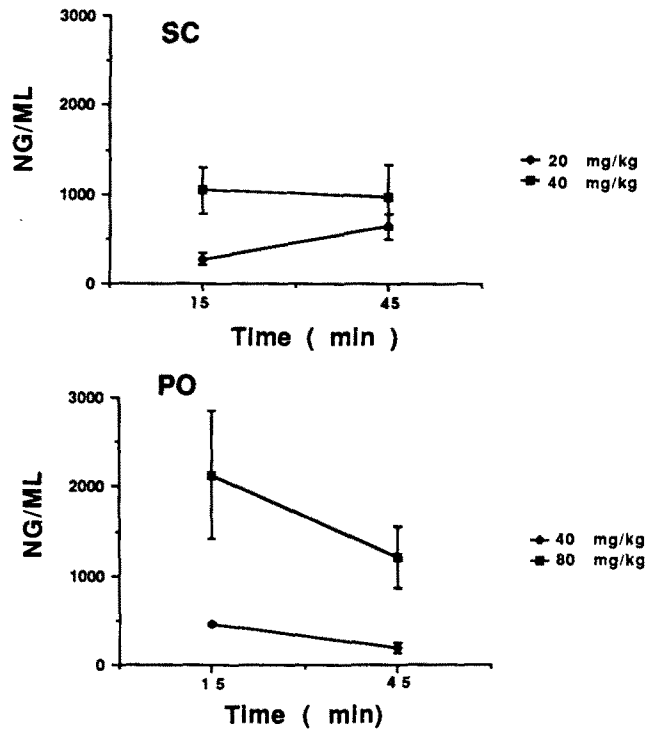

FIG. 6. Mean ± sem plasma cocaine levels (ng/ml) 15 and 45 minutes after the 16th daily cocaine injection for SC (top) and PO (bottom) groups. N=3–4 for each time point.

istration revealed several differences. While seizures leading to death were occasionally observed following 40 mg/kg by both PO and SC routes, six of the PO-80 group exhibited seizures without lethality. Previous reports have also documented seizures and death within this dose range following multiple cocaine injections (1, 4, 9, 11). A single administration of cocaine of the doses and routes used in the present study generally does not result in seizure or death, whereas multiple doses result in sensitization to a convulsive end point. We found that sensitization was similar with the PO and SC routes.

Both PO and SC cocaine were associated with an initial reduction in food consumption. In the PO group, food consumption returned to control values by approximately Day 3 and not until Day 7 in the SC group. Weight gain in the SC group is reduced compared to controls although this is not statistically significant. Weight gain in the PO-80 group was, however, significantly less than the controls. The anorexic effects of cocaine, as well as the development of tolerance to the anorexic effects, is well established (13,15). Also in the present study, SC administration was associated with an increase in water consumption on ml/kg body weight basis. Although this effect was not significant in the PO groups, and has not been previously reported, the possible effects of cocaine on water consumption should be taken into consideration.

Locomotor activity was found to be augmented by all doses of cocaine. Subcutaneous cocaine groups showed increases in locomotion which plateaued at Day 10 of drug administration while there were no differences between the SC-20 and SC-40 groups. As in SC administration, PO administration resulted in increased locomotion on subsequent test days with a plateau at Day 10. However, locomotor activity in the PO-80 group was generally higher than that in the PO-40 group providing a dose-related increase with oral administration.

In the present study, both PO and SC routes of administration produced the behavioral sensitization which is characteristic of chronic cocaine administration (5, 6, 9–11). Differences in the responses to SC versus PO administration were seen in the magnitude of the response. In general, the SC-40 group demonstrated up to 100% greater activity counts than the PO-40 group. While SC cocaine is associated with greater behavioral activation than PO, only PO administration showed dose-related increases in locomotion.

Like locomotor activity, stereotypy scores were intensified at all doses of cocaine. Subcutaneous groups reached maximal stereotypy scores on Day 5 of drug administration and there were no differences between the SC-20 and SC-40 groups. Stereotypy scores of the PO-80 groups also reached maximal levels on Day 5, however the scores of the PO-40 group increased through Day 15. Again, both PO and SC routes of administration produced sensitization to cocaine-induced stereotypy; an effect which is well documented (5, 6, 9,11). Unlike the higher levels of locomotor activity seen in the SC-40 group compared to the PO-40 group, stereotypy scores were similar in the two 40 mg/kg groups. However, as seen in locomotor activity, SC administration did not produce dose-response effects on stereotypy, whereas PO administration did.

Plasma cocaine levels showed different profiles based on the route of administration. While SC administration was associated with unchanged or increased concentrations with time, PO admin-

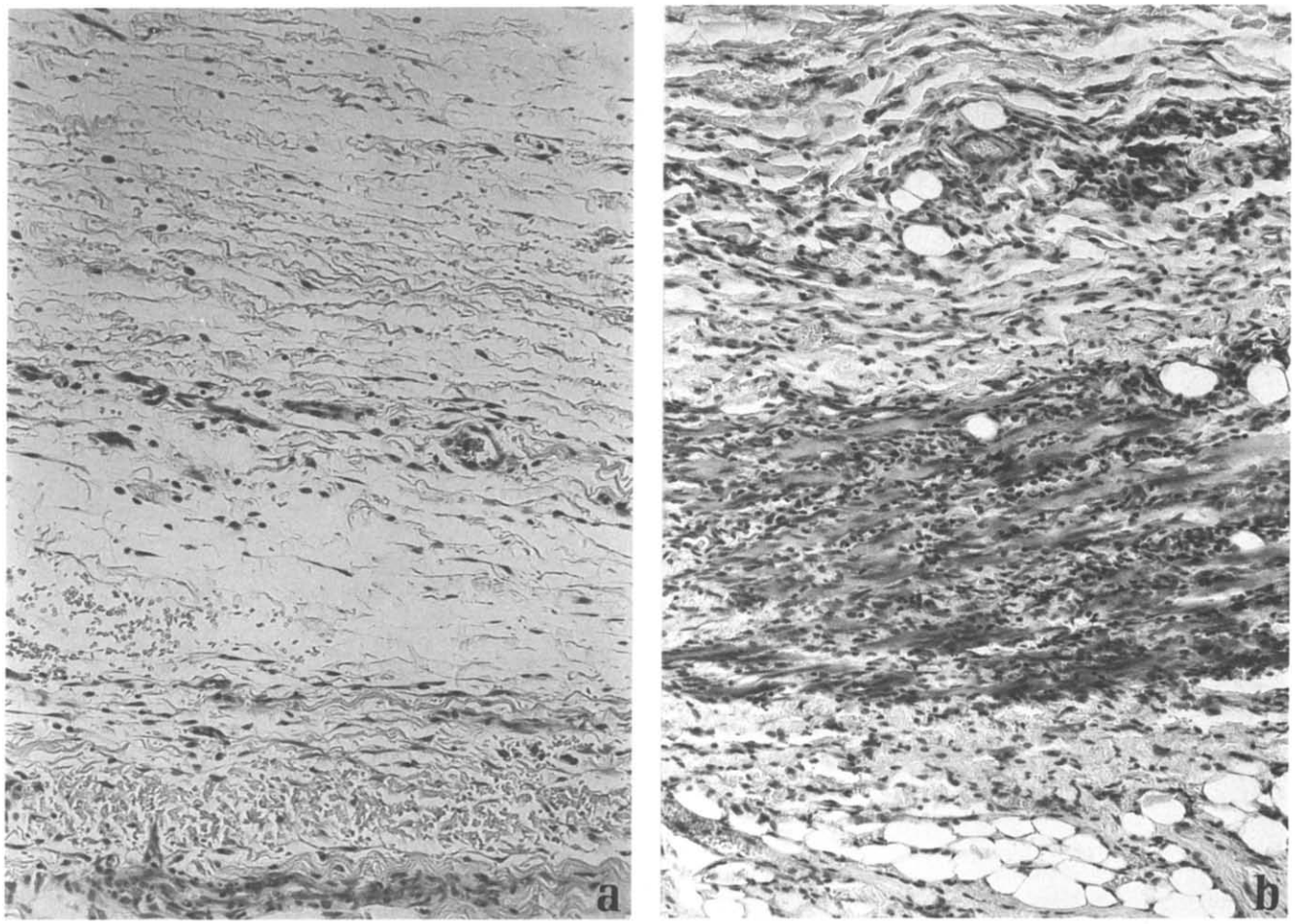

FIG. 7. Hematoxylin- and eosin-stained sections from the dermis of an animal in the SC-40 group (A) and one in the SC-0 group (B) after 16 consecutive days.

istration resulted in decreasing concentrations. Our SC-20 plasma concentrations are in agreement with the values in the literature as well as with the increase in plasma concentrations with time (7,8). In addition, Nayak and co-workers (8) have reported that SC administration of cocaine inhibits its uptake from the injection site, as 40% of the compound is present at the injection site after 30 minutes. This inhibition of uptake may relate to the increasing plasma levels seen in our study and that of Mule and Misra. To our knowledge, this report is the first to measure plasma cocaine concentrations in the rat following gastric intubation. Unpublished data from our laboratory show that the peak plasma concentration following PO administration is between 5 and 10 minutes in this dose range. Therefore, our PO data indicate that gastric intubation results in peak plasma concentrations substantially more rapidly than that reported following oral doses of cocaine in gelatin capsules in humans (12,14).

Histopathological analysis of skin patches from SC rats revealed severe dermal necrosis and inflammation of the subcutaneous tissue. Similar findings have been reported by Bruckner and co-workers (2) following acute SC cocaine injection. The combination of the vasoconstrictive effects of cocaine and the low pH may enhance the damage produced. However, neither epinephrine, a vasoconstrictor, nor an acidified saline solution produce lesions as severe as those produced by cocaine (2). PO administration of cocaine was not associated with any gross or histopathological changes of the esophagus, stomach or duodenum. Therefore, our data suggest that the GI tract tolerates the vasoconstrictive and acidic properties of the 0.4 and 0.8% solutions of cocaine used in the present study.

In conclusion, SC and PO routes both produce some toxicity, tolerance to anorexia, and behavioral sensitization. However, compared to SC administration on a mg/kg basis, PO was associated with less behavioral activation and lower plasma drug concentrations perhaps due to the rapid uptake and metabolism of cocaine administered by gastric intubation. The "depot effect" of SC administration appears to result in slow release of the drug, delayed peak plasma concentrations, and perhaps extended exposure of the brain regions associated with locomotion and stereotypic behaviors. For example, stereotypic behaviors at 40 mg/kg were maximal by the fifth day of SC injection and not until Day 15 following PO administration. The disadvantages of SC administration include the development of dermal lesions and the lack of dose-response relationships in this study. Since the oral route was not associated with any lesions and dose-response relationships were observed for several measures, PO should be considered in future studies utilizing chronic cocaine administration.

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