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Effect of Cocaine on the Production of Puberty-Accelerating Pheromone by Male Mice

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CHEN, C.-J. AND J. G. VANDENBERGH. *Effect of cocaine on the production of puberty-accelerating pheromone by male mice.* PHARMACOL BIOCHEM BEHAV 46(4) 835–839, 1993.—This study examined whether chronic cocaine exposure could reduce reproductive fitness of adult male mice by interfering with their production of the puberty-accelerating pheromone, an androgen-dependent urinary pheromone that accelerates puberty in juvenile female mice. Administered at a high dose of 60 mg/kg body weight/day, cocaine caused mortality, body weight loss, and suppression of circulating testosterone during the first week of treatment. However, at 40 mg/kg/day, it resulted in little adverse effect on these parameters. Animals showed habituation to repeated cocaine exposure by regaining part of the lost weight and reelevating suppressed testosterone level at later stages of treatment. Urine samples collected from animals receiving 60 mg/kg cocaine daily for 2 weeks lost the puberty-accelerating effect. However, neither a 3-day treatment of the same dose nor a lower dose of 40 mg/kg reduced the effectiveness. The diminished effect of cocaine-treated male mouse urine might reflect lowered testosterone levels with a lag of 10 to 15 days, similar to that of castrated male mouse urine. These results indicate that cocaine has no direct effect on the production of priming pheromone, and its metabolites in the urine did not affect the response of juvenile females to the pheromone.

Cocaine Mouse Puberty-accelerating pheromone Testosterone

COCAINE is a widely abused euphorigenic drug. As a psychomotor stimulant, it has addictive properties in humans and is readily self-administered by laboratory animals (3). The best characterized aspect of the pharmacological profile of cocaine is its inhibition of neuronal monoamine uptake mechanisms that result in heightened signals. Since both dopaminergic and adrenergic systems are known to play major roles in the neuroendocrine control of reproduction, it is important to examine the effect of chronic cocaine usage on all aspects of animal reproduction.

In humans, cocaine increases plasma prolactin and growth hormone levels and affects dexamethasone suppression of cortisol and thyroid-stimulating hormone response to thyroid-releasing hormone (7,8,11,12). In female rats, cocaine disrupts estrous cyclicity and normal rates of ovulation (6). In male rats, cocaine affects plasma prolactin, luteinizing hormone, and testosterone, and can lead to adrenocortical hypertrophy (2,13,17,19).

In mammals, a complex series of physiological and behavioral events must be orchestrated to ensure reproduction. A part of this orchestration results in puberty occurring at an appropriate time in an individual's development to promote maximum reproductive efficiency. Priming pheromones play important roles in regulating the onset of puberty in mice and other mammals (4,14,20–22). Juvenile female house mice are extremely sensitive to a puberty-accelerating pheromone found in adult male mouse urine. Male urine applied to the nose, at 0.03 ml per day for 8 days, induced a doubling of uterine weight (23).

The presence of puberty-accelerating pheromone in male mouse urine is androgen dependent. Urine of juvenile male mice fails to accelerate puberty (10), and adult males lose their capacity to accelerate puberty in females within 10 to 15 days after castration. A graded series of testosterone propionate doses given to castrate males restored pheromonal activity in a dose-dependent fashion (18). In addition, urine from dominant males resulted in the most pronounced acceleration of puberty, middle-ranking males had an intermediate effect, and urine of lowest-ranking males had no effect (9).

The ability of a male to produce social signals that modulate reproductive maturity of females is critical for reproductive success. While cocaine can affect testosterone levels in animals, it is important to determine if this effect is of physiological significance. In our laboratory, we found that cocaine injections, at daily doses of 30 or 40 mg/kg body weight during the period of male urine exposure, inhibited the responsiveness of female mice to puberty-accelerating pheromone (5). In the present study, we test whether cocaine administration affects the males' ability to produce puberty-accelerating pheromone. Correlations between pheromonal effectiveness and blood testosterone level, as well as weights of two androgen-related organs, testis and preputial gland, were noted.

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METHOD

All animals used were ICR albino mice. Males were proven breeders (9–12 months old) produced in our colony. Females were purchased from Charles River Laboratories at the weaning age of 21 days. Animals were housed singly in cages, and males and females were kept in separate rooms. They were maintained on a 14L : 10D cycle with lights on at 0600 and were provided with food and water ad lib.

For a period of 18 days, male mice in cocaine-treated groups received daily doses of either 40 or 60 mg/kg body weight cocaine hydrochloride (Sigma Chemical Co., St. Louis, MO) prepared with 0.9% saline. The daily dose was administered through two intraperitoneal injections, one given between 0800 and 0900 and the other between 1600 and 1700. Animals in the control group received injections of saline. Three hours after the morning injection, each animal was transferred into a urine collecting cage. Urine excreted during the following 3 h was collected and pooled with that collected from animals receiving the same treatment. The animal was then put back to its home cage and given the afternoon injection. Urine donors were started with 10 animals in the saline group, 10 in the 40 mg/kg cocaine group, and 20 animals in the 60 mg/kg cocaine group. However, only seven out of the 20 animals in the last group survived to contribute urine.

Pooled urine samples collected from cocaine-injected donors on days 3, 4, 5 and days 14, 15, 16 of injection and those collected on days 14, 15, 16 from saline-injected animals were tested for their effectivess in puberty acceleration. The bioassay was carried out by applying male urine to the external nares of juvenile female mice for 7 days starting at the age of 22 days. At the age of 29 days, these females were sacrificed and their uteri were removed. After trimming away fat and loose connective tissue, uterine weight was taken as an indicator of accelerated puberty (6,21).

Our bioassay used 90 juvenile female mice in six groups of 15 each. The first of these six groups was treated with saline as a control. The second group was treated with urine from saline-injected males (U-Saline). The next two groups were treated with urine from animals receiving 40 mg/kg cocaine; one used urine collected on days 3, 4, 5 (early sample, U-C40E) and the other used urine collected on days 14, 15, 16 (late sample, U-C40L). The last two groups were treated with urine samples collected from animals receiving 60 mg/kg cocaine (U-C60E and U-C60L).

On the 18th day of injection, male mice were sacrificed 3 h after the morning injection. Blood samples were collected by heart puncture for later measurement of testosterone concentration. The right testis and preputial glands were removed and weighed. To control for any "injection effect," a group of 12 male mice, randomly gathered from our pool of proven breeders, was also used as subjects. They received no injection but were sacrificed at the same hour of day as their conterparts. Their right testes and preputial glands were weighed and blood samples were collected.

When it appeared that the puberty-accelerating effect of male urine was decreased only after 2 weeks' cocaine injection at 60 mg/kg body weight/day, the second experiment was planned. In this experiment, we examined the effect of shorter-term, high-dose cocaine administration on blood testosterone levels and the androgen-related organs. Thirty more male mice were gathered from our proven breeder pool. They were also treated repeatedly with two daily cocaine injections of 30 mg/kg body weight each, but were sacrificed on days 1, 3, or 8. Seven animals out of these 30 died; thus data were gathered from 23 animals.

RIA

All serum samples were stored at −70°C until they could be assayed together. Samples from individual animals were processed in duplicate 50-μl aliquots. Testosterone concentrations were measured using a commercial radioimmunoassay kit for total testosterone (the "Coat-A-Count" solid-phase ^{125}I radioimmunoassay by Diagnostic Products Corporation, Los Angeles, CA). This assay has a detection limit of approximately 0.04 ng/ml.

Data Analysis

Analysis of variance (ANOVA) was used to analyze data from all experiments. Comparisons between values of different treatment groups were made using Duncan's New Multiple Range Test. Differences were considered to be significant at the 5% level ($p < 0.05$). All analyses and tests were performed using statistical routines in the SuperAnova software (Abacus Concepts, Inc., Berkeley, CA).

RESULTS

At age 29 days, juvenile female mice treated with male urine had heavier uteri than those of mice treated with saline. However, the uterine growth-promoting effect was suppressed in urine collected from male mice that had received cocaine at 60 mg/kg body weight/day for 14 days (U-C60L) (Fig. 1). Mean uterine weights of groups treated with other urine samples were more than double that of the saline-treated group. Nevertheless, only 40 mg/kg, days 14–16 urine samples and 60 mg/kg, days 3–6 urine samples resulted in uterine weights significantly higher than that of animals treated with U-C60L urine samples (Fig. 1).

Thirteen of the 20 male mice originally assigned to the 60-mg cocaine group died, but none of the animals receiving

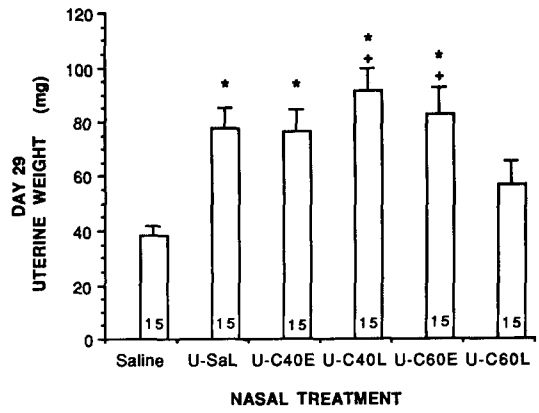

FIG. 1. Mean uterine weights of six groups of juvenile female mice treated with nasal application of saline (Saline) or adult male urine (U-). The urine samples were collected from male mice receiving daily injections of saline (Sa) or cocaine (C40: animals receiving cocaine at 40 mg/kg body weight/day; C60: animals receiving cocaine at 60 mg/kg/day). Urine samples collected on days 3, 4, and 5 were coded (E); urine samples collected on days 14, 15, and 16 were coded (L). *Different from saline-treated. +Different from U-C60L-treated.

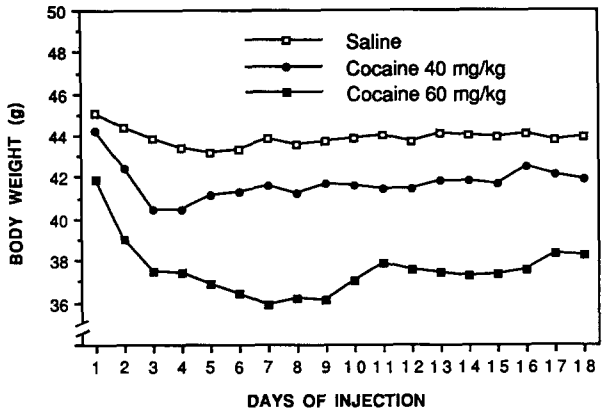

FIG. 2. Body weight changes of male mice receiving daily injections of saline or cocaine.

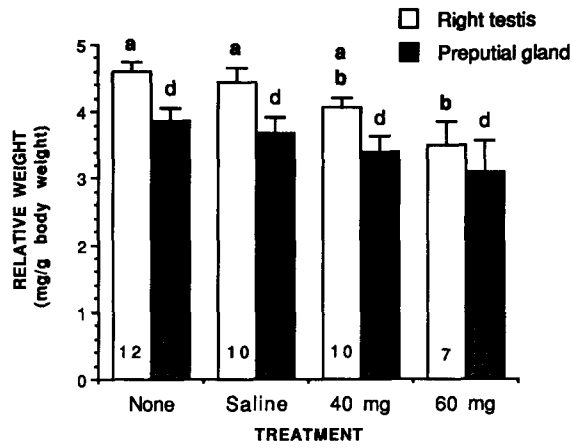

FIG. 3. The relative weights of right testes and preputial glands of male mice sacrificed on day 18. The animals were either not injected (None), received two daily injections of saline (Saline), received two daily injections of cocaine at 20 mg/kg each (40 mg), or received two daily injections of cocaine at 30 mg/kg each (60 mg). Columns sharing the same character are not significantly different from one another.

40 mg cocaine died. Most deaths occurred on the second or the third day of injection. Daily injections, cocaine or saline, resulted in weight loss in many animals, but the variation among individuals of the same group was large and group differences were not statistically significant. Animals receiving cocaine lost weight sharply during the first 3 days of treatment. Those in the 40-mg cocaine group started to regain weight on day 4, and those in the 60-mg cocaine group started on day 9. By day 18, net weight losses were 5.1% and 8.1% of their relative day 1 weights for these two groups, and 2.4% of its day 1 weight for the saline-injected group (Fig. 2).

Serum samples prepared from male mice that had received injections of either saline or cocaine for 18 days showed large intragroup variation of testosterone concentrations (Table 1). Variability in testosterone concentrations was also observed in samples collected from uninjected mice. The mean testosterone concentration of the 60-mg cocaine group was 46.7% lower than that of saline-injected controls, but group differences were not significant.

Measured on day 18, both the right testis and preputial glands of animals injected with cocaine were significantly lighter than those of animals injected with saline or not injected at all. However, when organ weights were calculated in relation to body weights, group differences in preputial gland weight were not significant (Fig. 3). The mean right testis weight of the 60-mg cocaine group, but not that of the 40-mg cocaine group, was significantly lower than that of either the saline control or uninjected control (Fig. 3).

At 60 mg/kg body weight/day, cocaine caused strong short-term effects. Mean body weights of animals sacrificed on day 3 and day 8 were 84% and 82%, respectively, of day 1 weights, while that of animals sacrificed on day 18 was 92% of day 1 weight. Serum testosterone concentrations of blood samples collected on days 1, 3, and 8 were uniformly low, ranging from 0.14 to 2.92 ng/ml. Although the day 3 group was the only one showing mean testosterone concentration significantly lower than that of the day 18 group, mean testosterone concentrations of samples collected on these 3 days were all significantly lower than that of blood samples collected from uninjected mice (Fig. 4).

There appeared to be a trend of testis weight decrease following repeated administration of cocaine at 60 mg/kg body weight/day, but not preputial gland weight (Fig. 5). No correlation was found between changes of the body weight and changes of either testis weight or preputial gland weight; thus, absolute weights of these organs were used for analyses. Given 60 mg/kg daily dose, animals sacrificed on days 1, 3, and 8

TABLE 1

SERUM TESTOSTERONE CONCENTRATIONS OF ADULT MALE MICE

Treatment	*n*	Range (ng/ml)	Mean Value	SE
No injection	12	0.26–21.98	7.020	2.299
Saline	10	0.19–20.99	7.179	2.676
Cocaine (40 mg/kg/day)	10	0.19–17.18	6.300	2.351
Cocaine (60 mg/kg/day)	7	0.19–16.33	4.117	2.647

Blood samples were collected from the saline-injected and the cocaine-injected animals at 3 h after last injections on day 18. The uninjected controls were sacrificed at a comparable time of the day.

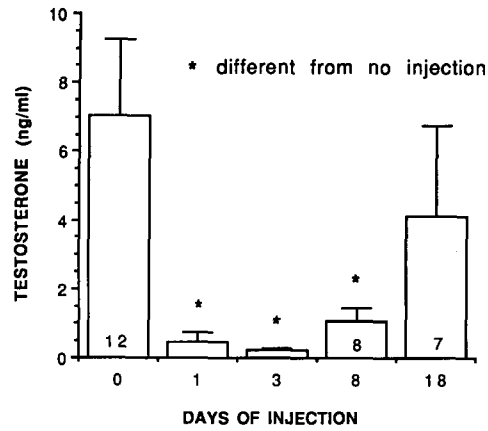

FIG. 4. Mean serum testosterone concentrations of five groups of male mice. The first group received no injection. The others received daily cocaine injections at a dosage of 60 mg/kg/day for 1, 3, 8, or 18 days. The data were pooled from two experiments.

all had mean testis weight higher than that of animals sacrificed on day 18 (Fig. 5). There was no group difference among mean preputial gland weights of animals sacrificed after 60 mg cocaine treatment of different durations.

DISCUSSION

In this study we tested whether chronic cocaine exposure interferes with the ability of adult male mice to produce the puberty-accelerating pheromone. We found that the priming effect of male mouse urine was decreased by repeated administration of cocaine at a very high dose—60 mg/kg body weight/day, for 2 weeks. At this dose, cocaine caused mortality and significant weight loss during the first week of treatment. In addition, blood samples collected on days 1, 3, and 8 showed suppression of serum testosterone levels. Nevertheless, blood samples collected on day 18, from cocaine-injected animals as well as controls, showed high variation for serum testosterone concentrations.

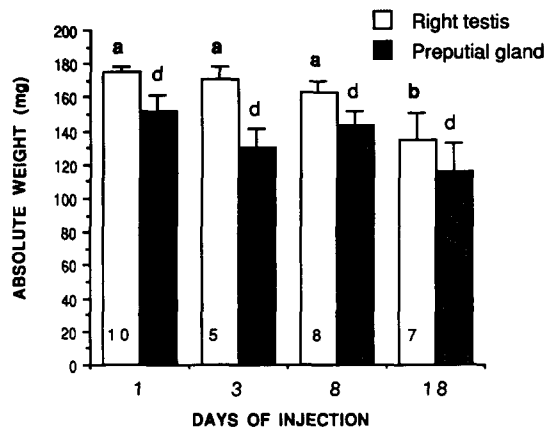

FIG. 5. The organ weights of right testes and preputial glands of male mice that received daily cocaine injections at a dosage of 60 mg/kg/day. Animals were sacrificed on day 1, 3, 8, or 18 of treatment. Columns sharing the same character are not significantly different from one another. The data were pooled from two experiments.

There are reports that cocaine interferes with the reproductive physiology of female animals (15,16). At the daily dose of 40 mg/kg body weight, cocaine inhibited the responsiveness of juvenile female mice to the puberty-accelerating pheromone and jeopardized their weight gain as well as normal uterine development (5). However, in our study, administration of cocaine at a daily dose of 40 mg/kg for more than 2 weeks brought about no adverse effect on the male's ability to stimulate early onset of puberty in females.

A prominent adverse effect of short-term, 60 mg/kg body weight cocaine treatment on male mice involves body weight. Animals sacrificed on day 3 and day 8 showed significantly lower terminal/beginning weight ratio than that of animals sacrificed on day 18. In addition, daily weight records of animals in the first experiment showed that all animals receiving injections (saline, 40 mg cocaine, or 60 mg cocaine) regained some weight after an initial drop. This phenomenon was also observed in cocaine-treated male rats (1). The first 3 days of cocaine treatment seems to be a critical period, because most mortality occurred on these days. Animals receiving 40 mg/kg cocaine started recovery of body weight after 3 days, but it took 5 more days before animals receiving 60 mg/kg cocaine started to recover.

Acute cocaine treatment of male rats produced an initial increase followed by a decrease in the plasma level of testosterone (2,13). Berul and Harclerode (2) reported that, at a dosage of 40 mg/kg for 15 days, cocaine lowered testosterone levels of blood samples collected at 2 h after injection but had no effect on testes weight. Longer-term administration of cocaine, at a dosage of 30 mg/kg for 72 days, resulted in a decrease in body weight, increase in locomotor activity, but no difference in testosterone level or relative testis weight of rats (1).

Since the cocaine-induced change of serum testosterone concentration varies according to the time of sampling, we collected all blood samples at 3 h after the last injection. Animals that did not receive cocaine injection were sacrificed at the comparable time of day. Results showed that, after only two injections of 30 mg/kg cocaine, testosterone levels of male mice dropped to a uniformly low level, around 0.25 ng/ml. Then, on day 8 of cocaine treatment, testosterone levels became more variable. On day 18, the range of serum testosterone concentrations of male mice receiving cocaine was close to that of animals receiving either saline injection or no injection. This is another indication that male mice developed habituation to repeated cocaine exposures.

Cocaine administered at 60 mg/kg/day for 18 days resulted in hypotrophy of testes but had no significant effect on the preputial glands. Testicular weight decrease proceeded slowly with repeated cocaine treatment. It did not follow the pattern of body weight change and did not correspond to changes of serum testosterone concentrations. Thus, chronic cocaine treatment at a high dose may have a gonadal effect, but the shorter-term effect of cocaine on testosterone may be a transient inhibition of secretion and/or synthesis.

The presence of puberty-accelerating pheromone in male mouse urine is androgen dependent, continuing for 10 to 15 days after castration (18). Thus, it is not surprising that urine samples collected during days 3 to 5 of 60 mg/kg cocaine administration were fully effective in promoting uterine development of juvenile female mice while those collected between days 14 and 16 were not. However, our results also indicate that cocaine has no direct effect on the production of priming pheromone, and its metabolites in male urine did not affect responses of juvenile females to the pheromone.

It is interesting to learn that chronic, high-dose cocaine treatment has only a limited effect on the reproductive fitness of adult male mice. While, at 60 mg/kg/day, cocaine caused hypotrophy of testes and transient inhibitions of body weight as well as serum testosterone level, animals demonstrated habituation to repeated cocaine administration. There was a great deal of variability among responses of individual animals. Many animals died on the second and the third days of treatment, but some of those that survived did not even lose weight. At a lower dose of 40 mg/kg, the significance of cocaine effects could not be established due to high level of variabilities. The habituation and the individual variability may very well be the source of some of the controversy on the adverse effects of cocaine on animals.

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