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# Cocaine Attenuates Puberty Acceleration in Female House Mice

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CHEN, C.-J. AND J. G. VANDENBERGH. *Cocaine attenuates puberty acceleration in female house mice.* PHARMACOL BIOCHEM BEHAV **44**(2) 281–285, 1993.—The onset of puberty in female house mice is advanced by exposure to a male urinary pheromone. This study tested whether cocaine could modify the juvenile female mouse's response to this pheromone. Puberty acceleration, as measured by uterine weight change, is inhibited by daily SC administration of 30 or 40 mg/kg body weight cocaine HCl between 20 and 26 days of age. Two daily injections of 20 mg/kg cocaine reduced both uterine development and body weight gain. Thus, cocaine may reduce an animals' reproductive fitness by isolating it from its social environment. At higher doses, cocaine can delay body growth, as well as the onset of puberty.

Cocaine Puberty Pheromone Female Mice

COCAINE is a euphorigenic drug that is widely abused. As a psychomotor stimulant, it has addictive properties in humans and is readily self-administered by laboratory animals (3). Many studies have revealed the ability of cocaine to influence and reinforce behavior (2). One of the best characterized aspects of the pharmacological profile of cocaine is its inhibition of neuronal monoamine uptake mechanisms. Cocaine seems to mimic the effects of norepinephrine and dopamine through blocking their reuptake by presynaptic neurons, thus causing heightened signals. Cocaine is also a potent local anesthetic. Some of the toxic effects of cocaine in humans have been attributed to its sympathomimetic effects while other toxic effects have been attributed to its local anesthetic effects (25).

While brain dopaminergic and adrenergic systems have been shown to play a major role in the neuroendocrine control of reproduction, knowledge concerning the long-term effects of cocaine usage on reproductive physiology is limited. In humans, cocaine increases plasma prolactin (PRL) and growth hormone levels and affects the dexamethasone suppression of cortisol and the thyroid-stimulating hormone response to thyroid-releasing hormone (6,9,10). In rats, cocaine affects plasma PRL, luteinizing hormone (LH) and testosterone and can lead to adrenocortical hypertrophy (1,11,18,30,31). Recently, it was reported that cocaine disrupts estrous cyclicity and alters the reproductive neuroendocrine axis in the rat (17). Thus, it is likely that cocaine can have an adverse effect on other stages of mammalian reproduction.

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 an important role in regulating the onset of puberty in mice and other mammals (4,12,32,33,35). Juvenile female house mice are extremely sensitive to a puberty-accelerating pheromone found in adult male urine, requiring only 3 successive days of exposure any time between 21 and 29 days of age to show accelerated puberty (5). Male urine applied to the nose of these mice, at 0.03 ml/day for 8 days, induces a doubling of uterine weight (36).

The ability of an animal to respond to social signals that modulate its reproductive performance is important for reproductive success. In this study, the puberty-accelerating effect of adult male urine was tested on cocaine-injected juvenile female mice to determine if this drug of abuse could interfere with animals' responsiveness to the social signals from their conspecifics.

## METHOD

All animals used were Swiss Webster albino mice produced in our breeding colony. They were maintained on a 14 L : 10 D cycle with lights on at 0600 h and provided with food and water ad lib. Animals weighing between 12–17 g were weaned at 20 or 21 days of age and randomly assigned to treatments. Each weaned animal was kept individually in a mouse cage and housed in a room containing of female mice only. Treatment started on the weaning day and lasted for 6 days. Animals were sacrificed late morning of the day following the last treatment.

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*Experiment 1*

This experiment was designed to investigate the effect of cocaine on the response of juvenile female mice to the puberty-accelerating pheromone contained in adult male urine. It involved a total of 60 animals randomly assigned to two groups. Between 21 and 26 days of age, animals in the experimental group were injected SC with twice-daily doses of 20 mg/kg body weight cocaine HCl (Sigma Chemical Co., St. Louis, Mo.) prepared with 0.9% saline. The first injection was given between 0800 and 0900 h and the second between 1600 and 1700 h. Animals in the control group were not injected. Starting from the second day of injection, 30–60 min after the morning injection half the animals in the control group and half in the experimental group were given saline on their nares while the other half were given urine collected from adult male mice. Animals were sacrificed at 27 days of age and their uteri removed. After trimming away the fat and loose connective tissue, the uterine weight was taken as an indicator of accelerated puberty (5,33).

*Experiment 2*

This experiment extended the first experiment by examining the dose dependency of the cocaine effect. Ninety-six animals were randomly assigned to 4 injection groups. Starting at 20 days of age, each group was given one of four SC injections in the morning: a) saline, 10 ml/kg body weight; b) cocaine, 10 mg/kg body weight; c) cocaine, 20 mg/kg body weight; and d) cocaine, 30 mg/kg body weight. After injection, half the animals receiving the same injection were given saline on their nares while the other half were given urine collected from adult male mice. All animals were sacrificed at 26 days of age and their uteri weighed.

*Data Analysis*

Analysis of variance (ANOVA) was used to analyze data from both experiments. Comparisons between the mean uterine weight for different treatment groups were made using Duncan's new multiple-range test. Differences were considered significant at the 5% level ($p < 0.05$). All analyses and tests were performed by using statistical routines in the SuperAnova software (Abacus Concepts, Inc., Berkeley, CA).

## RESULTS

*Experiment 1*

The puberty-accelerating effect of urine collected from adult male mice was confirmed in the control group. Exposure to male urine significantly enhanced the uterine growth of juvenile females in this group, but among animals injected with two daily doses of 20 mg/kg body weight cocaine for 6 days the effect of male urine was completely abolished (Table 1). Animals in the experimental group given nasal application of either saline or male urine showed lower mean uterine weight than that of saline-treated animals in the control group. In addition, the average weight gain of cocaine-injected animals was significantly lower.

*Experiment 2*

Male urine exposure induced uterine hypertrophy in juvenile, female mice. In the saline-injected group, the mean uterine weight of animals treated with male urine was significantly higher than that of animals treated with saline (Fig. 1). However, the male urine-promoted uterine growth was not statistically significant in any of the three cocaine-injected groups. An examination of the injection effect on uterine development revealed that among animals exposed to male urine those injected with 30 mg/kg body weight cocaine showed significantly lower mean uterine weight in comparison with that of animals injected with saline. However, among animals exposed to saline none of the injection doses induced significant differences in the mean uterine weights. There were no significant differences in body weight gain among treatment groups.

## DISCUSSION

This study tested whether cocaine administered to juvenile female house mice influenced the acceleration of puberty normally induced by adult urine applied to the nose. The results show that prepubertal daily injections of cocaine attenuated the effect of the puberty-accelerating pheromone contained in male urine. The inhibition was significant at the doses of 30 and 40 mg/kg body weight. At 40 mg/kg, cocaine reduced both body weight gain and uterine growth. However, doses of 30 mg/kg or lower had no effect on the uterine weight or body weight.

There are two routes through which cocaine may have an effect on the response to the puberty-regulating pheromone: a) a local anesthetic effect on the vomeronasal organ (VNO) due to the injected cocaine in circulation and b) the modulation of neuroendocrine systems regulating release of reproductive hormones.

The vomeronasal system has been implicated as the receptor for a number of priming pheromones (24). It serves as the receptor for the puberty-accelerating pheromone by juvenile

TABLE 1

EFFECT OF COCAINE (20 mg/kg BODY WEIGHT SC, TWICE A DAY FOR 6 DAYS) ON THE UTERINE DEVELOPMENT OF JUVENILE FEMALE MICE

| Cocaine Received (mg/kg) | Nasal Treatment | *n* | Uterine Weight (mg) | Body Weight (g) | |
|---|---|---|---|---|---|
| | | | | 21 days | 27 days |
| 0 | Saline | 15 | 65.03 ± 9.57 | 16.56 ± 0.19 | 20.55 ± 0.29 |
| 0 | Urine | 15 | 103.95 ± 8.96* | 16.43 ± 0.28 | 21.24 ± 0.40 |
| 40 | Saline | 15 | 40.29 ± 8.24† | 17.00 ± 0.27 | 19.04 ± 0.32† |
| 40 | Urine | 15 | 36.71 ± 5.59† | 16.82 ± 0.87 | 18.49 ± 0.31† |

Values are mean ± SE.

*$p < 0.05$ vs. saline treatment of the same group.

†$p < 0.05$ vs. the same treatment in the control group.

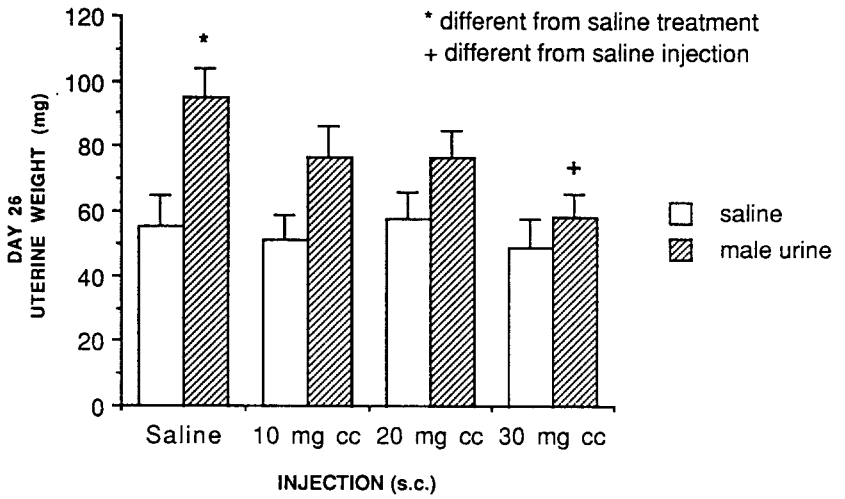


FIG. 1. Mean uterine weights of juvenile female mice injected with saline or 10, 20, and 30 mg/kg body weight cocaine, followed by nasal application of either adult male urine or saline. Error bars represent SEMs. $n = 12$ for all treatment groups. $*p < 0.05$ vs. saline treatment of the same group; $\dagger p < 0.05$ vs. urine treatment of the saline-injected group.

females (14,21). Vomeronasalectomy blocks the puberty-accelerating effects of male urine, as well as other pheromonal effects on ovarian function in mice such as the production of puberty-delaying pheromone by group-housed females (20) and the estrous-suppression effect of soiled bedding from grouped females (27). Little is known about how the signal is received by the VNO. Neurons of the VNO project to the accessory olfactory bulb, which projects to the corticomedial amygdala (29). Among the targets of corticomedial amygdaloid projections are the preoptic area (POA) and medial basal area (MBH) of the hypothalamus (19,29), centers known to regulate gonadotropin-releasing hormone (GnRH) release. This pathway provides the pheromones with the potential to regulate GnRH-containing perikarya in the POA and their axons and terminals in the MBH. Cocaine is a potent local anesthetic and thus may act directly on the sensory nerve terminals of the olfactory system, in particular the VNO, as well as on the afferent fibers en route. A local anesthetic effect seems an unlikely possibility, however, because only low levels of circulating cocaine would reach the sensory epithelium of the main and accessory olfactory systems.

The possibility that cocaine may have its effects through modulating neuroendocrine events related to puberty is supported because both high- and low-affinity sites for [$^3$H]cocaine binding are found in the striatum, hippocampus, olfactory tubercle, and hypothalamus of rats (13). The reuptake blocking ability of cocaine upon catecholamines and serotonin at these sites may be involved with inhibiting the puberty-accelerating effects of male urine. In particular, the brain opioid and hypothalamic catecholaminergic systems have been proposed to be involved in activating the sequence of neuroendocrine events necessary for puberty induction (37). Recently, Dluzen et al. (8) found that treating juvenile female mice with partially purified male urine extract resulted in a significant increase in $K^+$-stimulated dopamine release from the MBH. Thus, MBH can be a site where cocaine can modulate the effect of the puberty-accelerating pheromone. Such changes in MBH tuberoinfundibular dopaminergic (TIDA) activity will alter PRL release from the pituitary. The work of Keverne (15) suggests that priming pheromones have the common endocrine effect of modifying PRL secretion. Prolactin has also been specifically implicated as the primary endocrine change in puberty acceleration. For example, lowering PRL by injections of the dopamine agonist bromocriptine advanced the onset of puberty in intact and vomeronasal organ-lesioned females (21). In addition, exposure of female mice to males results in a rapid decrease of serum PRL (28).

While a clear link exists among priming pheromones, PRL secretion as controlled by the TIDA system, and puberty acceleration, it remains to be determined how, or if, cocaine may be involved in altering this specific cascade of events. Because the major putative action of cocaine is to prevent the reuptake of catecholamines, it would seem that increased levels of TIDA could be available and therefore puberty should actually be accelerated with cocaine. The present results indicate no evidence of puberty acceleration in cocaine injected in females receiving nasal saline treatment (Fig. 1). As the TIDA system has diminished reuptake capacity, it is not clear exactly how cocaine may be altering hypothalamic catecholamine function. Cocaine may either be exerting minimal effects upon this system or acting at different sites to produce this apparently anomalous result.

There are data that support a mediating role for PRL in the complex and divergent effects of cocaine upon the endocrine system. For example, hyperprolactinemia is often associated with chronic cocaine abuse in humans and persists during the abstinence from cocaine (6,22,23). It is found to be associated with suppression of LH secretory activity (22). Moreover, in ovariectomized rats acute cocaine injection of 10 or 20 mg/kg decreased serum PRL 1–3 h postinjection, while 40 mg/kg failed to change PRL secretion significantly (31). An alternative requiring more examination is that large doses of cocaine that induce seizure-like activity in rats are stressful and may have caused stress-induced release of prolactin (7).

Because both cocaine and the puberty-accelerating pheromone of adult male mouse urine are known to modulate the

serum levels of LH and PRL, it is likely that cocaine counteracted the priming pheromone through the hypothalamic-pituitary-gonadal axis. A less likely alternative is that a local anesthetic effect of cocaine on the sensory nerve terminals in the VNO could explain the attenuation of the uterine growth-promoting effect of male urine. Additional experimentation may reveal the relative importance of these two mechanisms.

The results of these experiments indicate that cocaine can decrease the sensitivity of an animal's response to chemical cues from its social environment. Thus, in addition to any direct effects cocaine may have on reproductive functions, it could isolate an individual from its social environment and thereby reduce its reproductive fitness. In mice, olfaction is the primary sense coordinating social signals with reproductive events (34). In other animals and humans, other senses or combinations of senses are involved in the coordination of reproduction. Our results with mice suggest that it may be worthwhile to explore similar effects of cocaine on modulating reproductive responsiveness in other organisms.

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