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Cocaine and Benzoylecgonine in Serum Microsamples of Intact and Gonadectomized Male and Female Wistar Rats

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VAN HAAREN, F., M. GARCEA, K. G. ANDERSON AND I. R. TEBBETT. *Cocaine and benzoylecgonine in serum microsamples of intact and gonadectomized male and female Wistar rats.* PHARMACOL BIOCHEM BEHAV **58**(2) 421–424, 1997.—Tail-tip plasma samples of intact and gonadectomized male and female Wistar rats were analyzed for cocaine and benzoylecgonine. The samples were obtained from immobilized subjects 10 and 30 min following the 1st and 22nd intraperitoneal injections of 10 mg/kg cocaine hydrochloride. Gender differences in plasma cocaine or benzoylecgonine levels were not observed after the first injection of cocaine because many of the samples were below the detection limit. Cocaine plasma levels were much higher after the 22nd injection, but gender differences were not observed either 10 or 30 min following cocaine administration. Plasma levels of benzoylecgonine were higher 30 min than 10 min after cocaine administration in intact and castrated male rats and ovariectomized female rats but not in intact female rats. These data show that, in rats, gender differences in cocaine metabolism may be observed after repeated cocaine administration, but the exact mechanism remains to be elucidated. © 1997 Elsevier Science Inc.

Acute and chronic cocaine administration Serum cocaine Serum benzoylecgonine Male and female rats

GENDER differences have been observed after cocaine administration. Female mice are more likely than male mice to die after a large dose of cocaine (1,2). The locomotor activity of female rats is acutely more responsive to cocaine than that of male rats, and sensitization occurs after lower doses (13,17), which is sometimes dependent on genetic background (3). In other experiments, the schedule-controlled behavior of female rats usually has been disrupted by lower doses of cocaine than that of male rats (15,16). Others have observed that female rats work harder than male rats to self-administer cocaine and that work rate is higher during the estrus than during the diestrus part of the estrus cycle (14). Thus, there appears to be ample evidence to support the contention that gonadal hormones modulate the behavioral effects of acute and/or chronic cocaine administration.

To understand better the gender differences in behavior after acute and chronic cocaine administration, it is important to evaluate the biological fate of cocaine after acute and chronic administration. Considerable gender differences in drug metabolism exist in rats, possibly as a result of the relatively high levels of an isozyme of cytochrome P450 in the 3A family (CYP3A) in male but not in female rats (4,6). The present experiment was designed to determine the plasma levels of cocaine and benzoylecgonine at 10 and 30 min following cocaine administration. These time points were chosen on the basis of the half-life of cocaine (approximately 30 min) and that of benzoylecgonine (approximately 40 min) and to correlate these half-lives with those at which the behavioral effects of cocaine administration are usually observed in intact and gonadectomized male and female Wistar rats [e.g., (15–17)].

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METHOD

Subjects

Eighteen male and 18 female Wistar rats were obtained from Harlan Sprague–Dawley (Indianapolis, IN) when they were approximately 60 days old. They were housed in group cages (3 same-sex subjects to a cage) upon arrival in the laboratory under a reversed 12-h light–dark cycle (lights on 6:00 PM) and constant temperature and humidity conditions. After 2 weeks, 9 of the male rats and 9 of the female rats were orchidectomized and ovariectomized, respectively; the remaining 9 male and 9 female subjects underwent sham surgery. All subjects were allowed 1 week to recover from surgery. Subjects then received only a limited amount of food (14 g/day per male subject, 12 g/day per female subject) for at least 2 weeks prior to the collection of the first plasma sample and throughout the experiment to simulate conditions under which subjects usually respond in behavioral experiments (5). Tap water was always available in the homecage. Subjects weighed an average of 435 g (intact males), 320 g (intact females), 449 g (castrated males), and 343 g (ovariectomized females) at the beginning of the experiment.

Procedure

All subjects received three intraperitoneal (IP) injections of physiological saline prior to the first IP injection of 10 mg/kg cocaine hydrochloride (1 ml/kg). The cocaine hydrochloride was obtained from the National Institute on Drug Abuse (Research Triangle Park, NC). Tail-tip blood samples were collected in heparinized tubes 10 and 30 min following the first cocaine injection, centrifuged and stored for subsequent analysis. Subjects were allowed to recover for 2 weeks and were then injected with 10 mg/kg cocaine on 21 consecutive days. Tail-tip samples were again obtained 10 and 30 min after the final (then 22nd) injection. To determine the levels of cocaine and benzoylecgonine, an accurately measured aliquot of serum (200 μl) was vortex mixed with 0.5 ml of 0.025 M potassium phosphate buffer at pH 3 and 10 μl of bupiracaine (100 μg/ml) as internal standard. This extract was then applied to a Strong Cation Exchange column (SCX) that had been conditioned under vacuum on a Vac Elut manifold (Varian, Harbor City, CA) with methanol (2 ml), water (1 ml) and 0.25 M phosphate buffer (1 ml). After application of the sample, the column was air dried for approximately 30 s and then washed with phosphate buffer (1 ml), 0.1 M acetic acid (0.5 ml) and methanol (1 ml). The column was again air dried for 30 s before eluting off the adsorbed drugs with ammoniacal methanol (3%, 2 ml). The final extract was evaporated to dryness under nitrogen and the residue reconstituted in 50 μl of methanol. A 20-μl aliquot of the extract was used for high performance liquid chromatography analysis. The analysis was performed using a Waters 510 pump (Milford, MA) to deliver solvent at 1.5 ml/min to a Spherisorb 5-μm ODS column (25 cm × 4.5 mm inner diamter). A Waters C18 Guard Pak precolumn was used to protect the analytical column. The detector was a Spectra Physics Focus multiwavelength detector (San Jose, CA) with an IBM Personal System/2 data system (Spectra Focus Software, Spectra Physics, San Jose, CA). The eluent was monitored at 230, 255 and 275 nm, and full spectra could be recorded from 190 to 400 nm for each peak. The mobile phase consisted of a 0.025 M potassium phosphate buffer: acetonitrile (85:15) with diethylamine 25 ml/L. (pH adjusted to 2.9 with concentrated orthophosphoric acid). Quantitative analyses were achieved by comparison of peak area with ex-

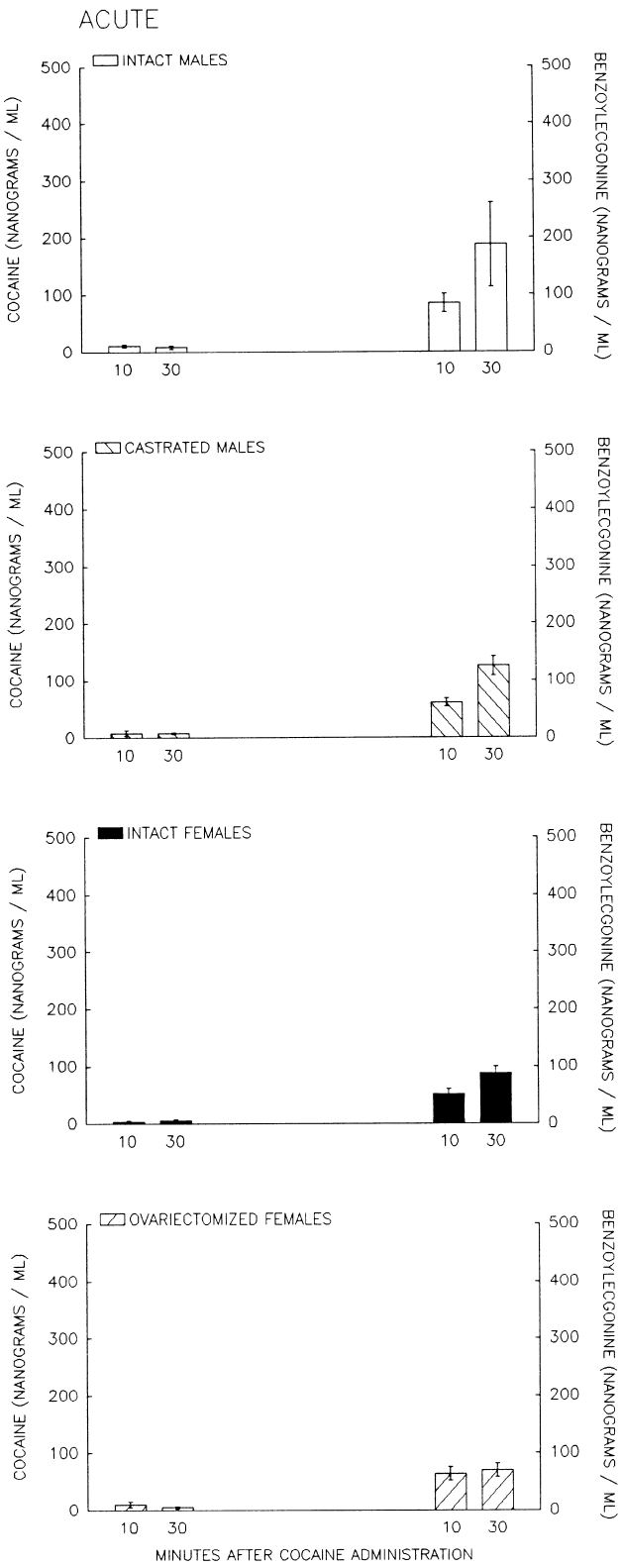

FIG. 1. Serum levels of cocaine (ng/ml, left-hand axis of each individual panel) and benzoylecgonine (ng/ml, right-hand axis of each individual panel) for intact male (top row), castrated male (second row), intact female (third row) and ovariectomized female (bottom row) Wistar rats 10 and 30 min following the first injection of 10 mg/kg cocaine (IP, 1 ml/kg).

tracted standards (detection threshold = 5 ng/ml). This procedure results in extraction recoveries of 83 ± 9% for benzoylecgonine and 93 ± 7% for cocaine. Each determination was taken as the mean of two replicate injections. The calibration graph was produced over the range of 0.01–5 μg/ml. Quantitation limits were 5 ng/ml for cocaine and benzoylecgonine.

RESULTS

Figure 1 shows serum levels of cocaine (ng/ml, left-hand side of each individual panel) and benzoylecgonine (ng/ml, right-hand side of each individual panel) for intact male (n = 8, top row), castrated male (n = 9, second row), intact female (n = 9, third row) and ovariectomized female (n = 8, bottom row) Wistar rats, obtained 10 and 30 min following the first cocaine injection. Figure 2 shows the same data obtained after the 22nd cocaine injection.

Figure 1 shows that cocaine and benzoylecgonine levels were very low 10 and 30 min following the first cocaine injection. In fact many of the data were below the quantitation threshold (5 ng/ml), in effect preventing an acceptable interpretation of these observations.

Figure 2 shows that the plasma levels of cocaine and benzoylecgonine after 21 additional daily cocaine injections were much higher than those observed after the first cocaine injection in all groups of subjects. These data were analyzed with an analysis of variance involving gender (male and female), gonadectomy (castration and ovariectomy) and time of observation (10 and 30 min following administration). This analysis showed that cocaine plasma levels were similar in all groups of subjects and were not different 10 or 30 min after cocaine administration. Benzoylecgonine plasma levels were higher 30 min following cocaine administration than 10 min following cocaine administration in intact and castrated male rats and ovariectomized female rats [$F(1, 30) = 38.91$, $p < 0.01$]. The benzoylecgonine plasma levels observed after 10 and 30 min were not different in intact female rats as confirmed by a significant Gender × Time of Observation interaction [$F(1, 30) = 10.53$, $p < 0.01$].

DISCUSSION

The results of thess experiments show that gender differences in plasma cocaine or benzoylecgonine levels could not be evaluated after the first injection of cocaine because many of the samples obtained 10 and 30 min after IP injection of 10.0 mg/kg cocaine hydrochloride were below the detection limit. Cocaine plasma levels were much higher 10 and 30 min after the 22nd daily injection, but gender differences were not observed. These results confirm observations by others who have previously shown that the bioavailability of cocaine is increased after repeated administration. The increased bioavailability may be related to the hepatotoxic effects of cocaine, which would compromise the first pass metabolism of cocaine in the liver following absorption from the gastrointestinal tract (10).

Plasma levels of benzoylecgonine were higher 30 min than

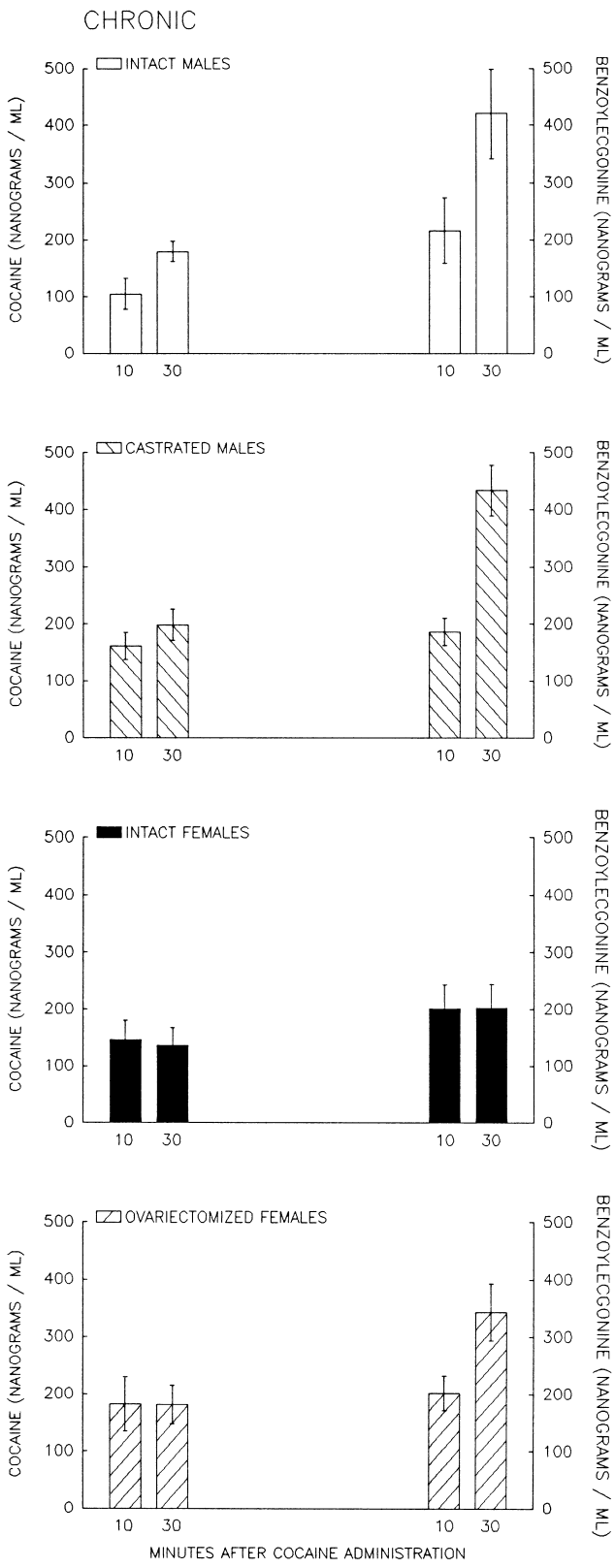

FIG. 2. Serum levels of cocaine (ng/ml, left-hand axis of each individual panel) and benzoylecgonine (ng/ml, right-hand axis of each individual panel) for intact male (top row), castrated male (second row), intact female (third row) and ovariectomized female (bottom row) Wistar rats 10 and 30 min following the 22nd injection of 10 mg/kg cocaine (IP, 1 ml/kg).

10 min after cocaine administration in intact and castrated male rats and ovariectomized female rats but not in intact female rats. Perhaps the difference is due to further metabolism of benzoylecgonine or its excretion in intact female rats. In any case, these results suggest that the metabolism of cocaine may be influenced by a subjects' hormonal status. Unfortunately, we did not assess the stage of the estrus cycle at the time of sample collection. This omission, however, does not appear to be crucial because the plasma levels of cocaine and benzoylecgonine after the first cocaine injection showed very little variability in intact female rats (or in any of the other groups of subjects). Repeated cocaine administration, as in the present experiment, disrupts estrus cyclicity and induces repetitive days of estrus and prolonged periods of diestrus in female rats (7,8). In those experiments, 10.0 mg/kg/day of cocaine produced circulating drug levels comparable to those in the present experiment (approximately 180 ng/ml) and permanently disrupted the estrus cycle (as determined by the number of proestrus:estrus events per 3-week period of analysis) in nearly 50% of the subjects even with cessation of treatment. These observations in conjunction with those of others who have reported that estradiol administration enhances behavioral sensitization to cocaine (11) suggest that female gonadal hormones such as estrogen and progesterone may play an important role in the metabolism and prolonged behavioral effects of cocaine in female rats. Because benzoylecgonine is formed by esterase-catalyzed hydrolysis of cocaine, esterase activity can be modulated by hormone levels. Indeed, pregnant women, fetuses and individuals with liver disease show lower cholinesterase activity, thus potentially increasing the risk of cocaine toxicity (9). Elevated levels of the pregnancy hormone progesterone may have been responsible for the increased toxicity of cocaine in pregnant women because others have shown that progesterone administration increases the cardiovascular toxicity to cocaine in nonpregnant ewes (12). These and other interesting possibilities should be scrutinized further to determine the full extent to which the behavioral and neuroendocrine effects of acute and chronic cocaine may be different in male and female organisms.

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