Cocaine-Induced Sex Differences in D1 Dopamine Receptor mRNA Levels after Acute Cocaine Administration

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Introduction: Although it is known that female rats have a more robust behavioral response to acute cocaine administration than male rats, the neurobiological mechanisms underlying these differences remain unclear. The purpose of the present study was to determine if there are sex differences in cocaine's regulation of dopamine D1 and D2 receptor mRNA levels.

Methods: Male and female Fischer rats received acute cocaine (20 mg/kg, intraperitoneal) or saline. Ambulatory activity was recorded one hour post drug treatment. Rats were then sacrificed either 1 or 24 hours post drug treatment and D1/D2 DA receptor mRNA levels were measured via solution hybridization assay.

Results: Cocaine-induced ambulatory activity was greater in female than male rats. There were no sex differences in baseline levels of D1 and D2 receptor mRNA in the caudate putamen (CPu) or the nucleus accumbens (NAc). Cocaine administration reduced levels of D1 mRNA in the NAc only in male rats.

Conclusion: Our findings suggest that the regulation of striatal D1 mRNA levels after acute cocaine administration is a sexually dimorphic process. We also hypothesize that the D1 receptor may be an important substrate in the regulation of sex differences in cocaine-induced locomotor activity. (*Ethn Dis*. 2010;20[Suppl 1]:S1-24–S1-27)

Key Words: Cocaine, Sex Differences, Locomotor Behavior, Dopamine, mRNA

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Introduction

As more attention is paid to sex specific effects on responses to drugs, it is becoming apparent that men and women react differently to cocaine. Women experience more nervousness after intranasal administration of cocaine, take longer to feel the subjective effects of cocaine, report less euphoria and dysphoria, have more severe drug use at intake, and report shorter abstinence periods than do men.[7,9] Taken together, these studies suggest that cocaine's effects are sex-dependent and women may be more susceptible or sensitive to cocaine's addictive properties than men. In rodents, sex differences are present at all of the phases of drug abuse, which include initiation, escalation of use, and adaptations and/or responses to chronic cocaine administration.[2]

The mesocorticolimbic dopamine (DA) system has been postulated to be the primary regulator of cocaine's psychomotor and rewarding effects.[5] Intrinsic sex differences in the DA system have been documented. For example, male rats have higher levels of D1 receptors in the nucleus accumbens (NAc) than do female rats,[1] although no sex differences in number or binding characteristics of striatal D2 receptors have been reported.[4,8] Female rats have a greater rate of DA release and re-uptake and higher DA transporter levels in the striatum than male rats.[16] In female rats, the nigrostriatal DA neurotransmission is more tightly regulated by autoreceptors and transporter mechanisms than in male rats, a difference that may be related to the greater autoreceptor control of DA in females.[17] Male rats have a higher rate of DA turnover in the caudate putamen (CPu) than do female rats, further suggesting sex differences in autoreceptor-mediated DA activity.[2,3] Furthermore, D1, but not D2, receptor antagonists block cocaine-induced 3-[(±)-2-carboxypiperazin-4-yl]-propyl-1-phosphonic acid (CPP) and locomotor responses with different efficiencies between sexes; D1 receptor antagonists block cocaine's effects with a lower dose range in female than male rats.[3,11] Although sex differences in baseline and cocaine-induced DA release and re-uptake dynamics, as well as DA-induced intracellular responses[10,12,20] have been established, how cocaine affects transcriptional responses, such as DA receptor mRNA levels, has not been well studied. To this end, we examined whether D1 and D2 DA receptor mRNA levels are sexually disparate at baseline or after cocaine administration or both.

Methods

Animals

Eight-week-old male (200–250 g) and female (150–200 g) Fischer rats purchased from Charles River (Raleigh, NC) were individually housed with free access to standard lab chow and water ad libitum –12-hour light/dark cycle (lights on at 9:00 am). Fischer rats were chosen because a large number of studies have shown their suitability for investigations of sex differences in cocaine-induced behavioral responses. Both sexes were housed in the same room to ensure identical housing conditions. Rats were handled and weighed for 3 days prior to experimental manipulations. As suggested by Walker et al,[15] the use of lavaged female rats could result in inaccurate behavioral responses

when making side-by-side comparisons to male rats. For this reason, animals were placed into groups at random without regard to estrous cycle. Two separate cohorts of rats were used ($n=5$ per group). Animal care was in accordance with the Guide for the Care and Use of Laboratory Animals (NIH, Bethesda, MD) and approved by the Institutional Animal Care and Use Committee of Hunter College.

Drug Treatment

Cocaine (dissolved in saline .9%) was injected (intraperitoneal) in the rat in its home cage 30 minutes after lights-on at a concentration of 20 mg/kg. This cocaine dose has been demonstrated to produce robust sex differences in cocaine-stimulated motor activity, without reaching a maximal effect in either sex.[3]

Behavioral Measurement

Ambulatory activities were monitored in the home cages with a Photobeam Activity System from San Diego Instruments (San Diego, CA) as previously described.[3] Ambulatory activity represents the number of counts produced by two consecutive photobeam interruptions in the horizontal frame. Behavioral activity was recorded for 60 minutes and is presented as mean sum of ambulatory counts ± standard error of the mean (SEM).

RNA Probes

D1 and D2 RNA probes were constructed from 1.5 kb *Apa*I-*Sal*I (D1) and 0.5 kb *Dsa*I-*Dsa*I (D2) cDNA fragments of the D1 and D2 receptor genes that had been previously subcloned into pGEM-T and pGEM -5Zf(-) vectors, respectively. Receptor clones were verified by sequencing (The Rockefeller University, NY, NY). The D1 receptor clone did not contain homologous sequences for the D5 receptor, and the D2 receptor clone did not contain homologous sequences for either D3 or D4 receptors. Procedures for synthesis of RNA probes have been described in detail elsewhere.[6] Templates for sense and antisense transcripts of the D1 and D2 receptor probes were prepared by digestion of plasmid DNA with the appropriate restriction enzymes. The riboprobes and unlabeled sense strands were transcribed from D1 and D2 plasmids in the presence of α-[^{33}P]-CTP using T7 and SP6 polymerase reverse transcription systems.

Solution Hybridization RNase Protection-Trichloracetic (TCA) Precipitation Assay

Rats were decapitated 1 or 24 hours after drug treatment. Brains were removed and coronal slices (1 mm) were cut out in a matrix as previously described.[6] The CPu and NAc were then dissected out (1.90 mm to 0.90 mm anterior to bregma) on a cold glass plate and frozen at −80°C. Tissues were homogenized in Trizol reagent and total RNA was extracted according to the manufacturer's instructions. mRNA was stored at −80°C until assayed. D1 and D2 mRNA levels were measured by solution hybridization assay as previously described.[6] In brief, 20-μL aliquots of total mRNA extracts were hybridized to ^{33}P-riboprobes in a hybridization buffer overnight. The mixture was then subjected to 40 μg/mL ribonuclease A and 2 μg/mL ribonuclease T1 for 1 hour. Samples were precipitated with TCA, and the dcms were counted by liquid scintillation. Comparisons were made with respective D1 or D2 receptor standard calibration curves to quantify levels of each respective mRNA. Samples are expressed in pg of hybridized mRNA normalized to the total μg of RNA.

Statistics

Ambulatory activity data were summed for each subject and are presented as mean ± SEM. D1 and D2 receptor mRNA levels are expressed as percent from control ± SEM. Two-way analyses of variance (ANOVAs) were used for analysis of ambulatory activity: cocaine (cocaine or saline) × sex (male vs female). Two-way ANOVAs were used to determine the effects of time and sex on baseline mRNA levels as follows: time (1 or 24 hours) × sex (male vs female). Tukey honestly significant difference tests were used for multiple comparisons when appropriate. We used *t*-test analyses to determine statistically significant differences between levels of D1 and D2 mRNA by comparison of cocaine-treated animals to their respective saline-treated controls. Significance in all cases was considered to be $P<.05$.

RESULTS

As shown in Figure 1, cocaine administration increased ambulatory activity in both male and female rats [$F(1,28)=57.23$, $P<.0001$]. Female rats had higher ambulatory activity than male rats both at baseline and after cocaine administration [$F(1,28)=4.29$, $P=.0477$; $P<.0005$]. Neither significant sex differences nor interactions between sex, drugs and time were observed in either baseline levels of D1 and D2 mRNA in the CPu and NAc at 1 or 24 hours post-treatment (data not shown). In the NAc of male rats, D1 mRNA levels were decreased 1 hour after cocaine administration ($t=2.58$, $P<.05$; Figure 2).

DISCUSSION

In a finding consistent with previous reports from our group and others,[2] female rats had higher ambulatory activity after an acute cocaine injection. Our findings are novel by demonstrating that dopamine receptor mRNA levels are differentially regulated between sexes after cocaine administration.

Although D1 mRNA levels in the CPu of male rats are altered after acute

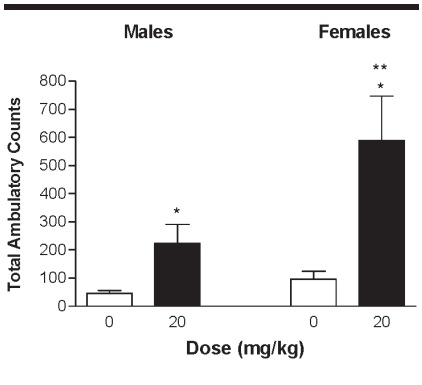

Fig 1. Sum of ambulatory activities in male and female rats (*n*=8 per group) after acute cocaine (20 mg/kg) or saline administration. Behavioral activity was recorded for 60 minutes and is presented as mean sum of ambulatory counts ± SEM. *Denotes a significant effect of cocaine. **Denotes a main effect of sex

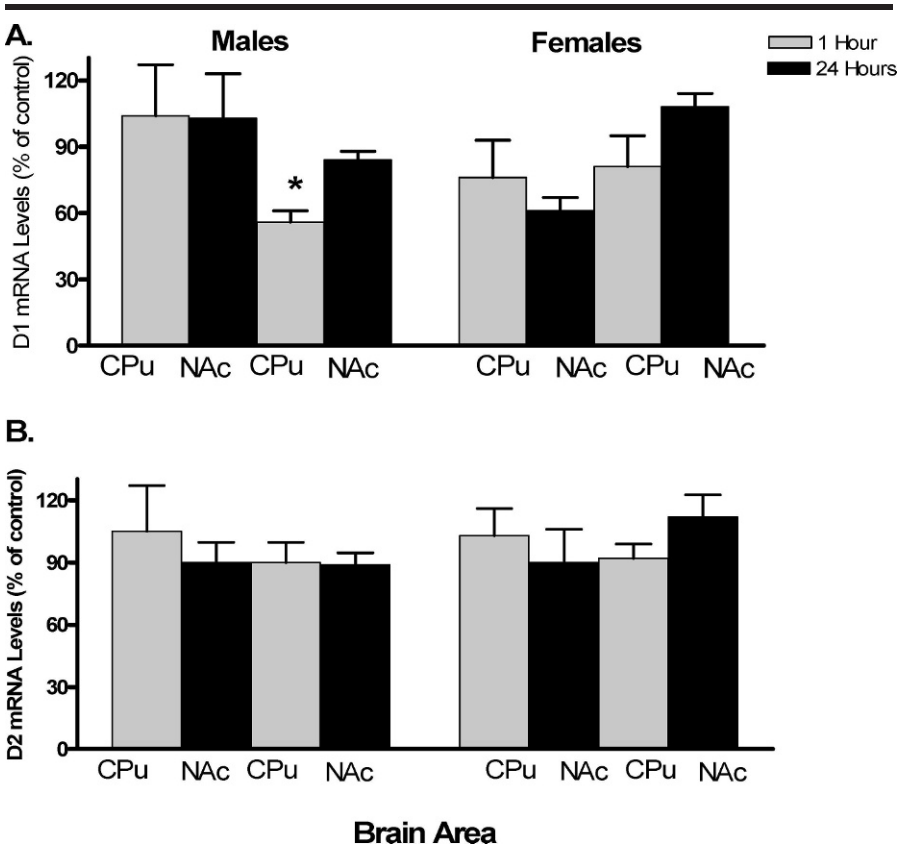

Fig 2. D1 (A) and D2 (B) mRNA levels in male and female rats 1 and 24 hours after acute cocaine (20 mg/kg) or saline administration are presented as percent of control from saline-treated rats. Levels after 1 hour of saline or cocaine treatment are denoted by gray bars; levels at 24 hours after saline or cocaine treatment are denoted by solid bars. *Denotes a significant effect of cocaine as compared with respective saline-treated groups ($P<.05$). All groups contained 5 animals

cocaine administration, the direction of this effect has not been consistent between studies. For example, Yuferov et al[18] demonstrated a significant increase in D1 mRNA levels in the CPu of male rats 30 minutes after binge pattern cocaine administration. Similarly, Svensson and Hurd[14] reported an increase in D1 receptor mRNA levels in the CPu 6 hours after a single injection of cocaine, whereas Schmidt-Mutter et al[13] did not find any changes 6 hours after single cocaine administration. Discrepancies between these studies and our own may be attributable to the manner of cocaine administration (binge vs single), the dose of cocaine used (Yuferov et al,[18] a total of 45 mg/kg; Svensson and Hurd,[14] 30 mg/kg; Schmidt-Mutter et al,[13] 20 mg/kg; and in this study 20 mg/kg) or the time of mRNA measurements (Yuferov et al,[18] 3.5 hours; Svensson and Hurd,[14] and Schmidt-Mutter et al,[13] 6 hours; and our study, 1 and 24 hours).

In female rats, a non-significant reduction of 40% was observed in D1 receptor mRNA levels in the CPu. D1 receptor density in the CPu has been shown to fluctuate with the estrous cycle,[8] and so estrous cycle effects may account for the increased variability observed in D1 mRNA levels and the lack of a significant effect in the current study. Moreover, our lab and others have shown that estrogen and progesterone replacement in ovariectomized females affects cocaine-induced alterations in preprodynorphin mRNA and other genes in the mesocorticolimbic DA system.[6,19] More detailed studies delineating cocaine's interaction with ovarian hormones in intact female rats are necessary to clarify these findings.

In the NAc, D1 receptor mRNA levels were significantly reduced in male rats 1 hour after cocaine administration. At 24 hours after drug treatment, however, transcript levels returned to that of saline-treated controls. To our knowledge, this is the first report showing sexually dimorphic molecular alterations in the striatum after acute cocaine administration. Jenab et al[6] demonstrated that in both male and female rats, the mRNA levels of the immediate early gene c-*fos* were increased after acute cocaine administration. On the other hand, preprodynorphin mRNA levels did not change in either male or female rats after acute cocaine administration.[6] Therefore, cocaine-induced alterations in D1 mRNA may play a vital role in either the observed sex differences in behavior or the initial neuronal adaptations in response to acute cocaine.

No alterations in D2 receptor mRNA in either male or female rats

were observed. We have previously demonstrated that D2 antagonist did not affect baseline activity nor produce sex differences in cocaine-induced motor behavior nor block cocaine-induced conditioned place preference.[3,11] Thus, the D2 receptor in the striatum may play only a limited role in the observed sex differences in cocaine-induced locomotion.

IMPLICATIONS FOR IMPROVING HEALTH DISPARITIES

Recent papers have pinpointed the D1 DA receptor-PKA signaling pathway as a contributor to females' hypersensitivity to cocaine.[10,12,20] Our results further support this postulate by showing that the D1 receptor may be an important substrate in the regulation of sex differences in cocaine-induced psychomotor and reinforcement effects. The extent to which differences in D1 translation contribute to the known sex differences in vulnerability to cocaine's reward effects and behavioral responses remains to be determined.

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