Sex Differences in Dopamine D_2-like Receptor-mediated G-protein Activation in the Medial Prefrontal Cortex after Cocaine

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Introduction: Sexually dimorphic behavioral responses to cocaine have been linked to a difference in activation of dopamine receptors. Our study was conducted to determine whether dopamine D_2-like receptor-activated G-protein contributes to sex differences in response to cocaine in the medial prefrontal cortex (mPFC).

Method: *In vitro* functional autoradiography was performed using dopamine receptor D_2 agonist (quinpirole, 100 μM) to stimulate [^{35}S]GTPγS binding in brain tissue sections from male and female Fischer rats treated with saline (1 mL/kg) or cocaine (20 mg/kg; i.p.).

Results: Overall, quinpirole increased G-protein activation in the caudate-putamen, nucleus accumbens, and frontal cortex in both sexes. Although saline-treated male rats had higher [^{35}S]GTPγS binding in the mPFC than their female counterparts, cocaine-treated females had higher [^{35}S]GTPγS binding in the mPFC than cocaine-treated males.

Conclusions: These data suggest that both intrinsic and activational effects of dopamine D_2-like receptor-mediated G-protein activation in the mPFC may contribute to the differences between males and females in their response to acute cocaine administration. (*Ethn Dis.* 2010;20[Suppl 1]:S1-88–S1-91)

Key Words: Cocaine, Dopamine, G-protein, Cortex

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Introduction

Cocaine abuse is widespread in Western countries. Behavioral and biochemical effects of cocaine are attributed to the elevation of dopamine synaptic levels and the subsequent activation of dopamine receptors in the mesocorticolimbic system.[1] Systemic administration of D_2-like receptor antagonists has been found to blunt the acute cocaine-induced locomotor augmenting and rewarding effects.[2,3] The dopamine D_2-like receptors are coupled to G-proteins (Gα i/o) and activate the G-protein transduction of the intracellular second messenger signaling necessary for inhibiting adenylyl cyclase. In reward areas, hypersensitivity to cocaine and other psychostimulants is associated with changes in the activation of the dopaminergic signaling and thus G-protein transduction. For example, acute and chronic cocaine administration alters G-protein expression and activation in the mesocorticolimbic system, [4,5] a fact that suggests D_2-like receptors and underlying G-protein may be critical for behaviors elicited by cocaine.

Evidence has shown a sexually dimorphic pattern in behavioral responses to cocaine. For example, behavioral activation and rewarding effect after cocaine administration are greater in female than in male rats.[6] An intrinsic sex difference in the mesocorticolimbic dopaminergic system has also been shown.[7,8] In the striatum, acute cocaine induces a higher dopamine efflux in females than in males.[8] Additionally, sexual disparity in D_2-like receptor agonist-induced locomotor behavior has been reported, thus indicating a difference in D_2-like receptor sensitivity.[6,9,10] Taken together, intrinsic differences in intracellular responses to cocaine mediated by D_2-like receptors may contribute to the known sexually dimorphic behavioral responses to cocaine. Because G-protein activation is essential to modulate dopamine-mediated cellular responses associated with cocaine-induced behavioral and reward activities such as the adenylyl cyclase-PKA signaling pathway, this crucial cellular mediator may underlie some sex differences in cocaine responses. Thus, the purpose of this study was to investigate whether the D_2-like receptor activated G-protein levels after cocaine administration are sexually dimorphic. To this end, the [^{35}S]GTPγS binding assay was used as an indictor for D_2-like receptor agonist-activated G-protein levels in specific areas of the mesocorticolimbic system of male and female rats.

Methods

Animals

Eight-week-old male and female Fischer rats were purchased from Charles River Laboratories (Kingston, NY). Animals were individually housed in cages with free access to food and water and were maintained on a 12-hour dark-light cycle (light on at 9 AM). Previous studies have demonstrated that repeated vaginal lavage alters behavioral response to cocaine and increases dopamine release in the striatum. [11] Thus, female rats were randomly assigned into groups without regard to estrous cycle. All experiments were carried out in accordance with NIH Guidelines for the Care of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at Hunter College.

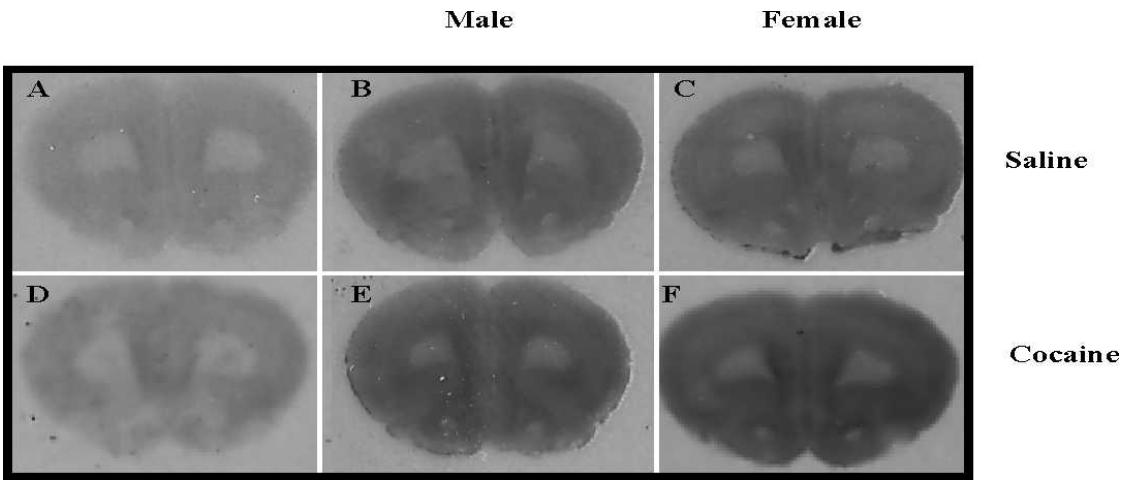

Fig 1. Representative autoradiograms of quinpirole-stimulated [^{35}S]GTPγS binding in the medial prefrontal cortex (mPFC). Basal binding of saline- and cocaine-treated rats (A and D, respectively), quinpirole-stimulated binding in saline- and cocaine-treated male rats (B and E, respectively), and quinpirole-stimulated binding in saline- and cocaine-treated female rats (C and F, respectively)

Drugs, Chemicals, and Cocaine Administration

Cocaine hydrochloride, guanosine 5′-O-(3-thiotriphosphate) (GTPγS), guanosine 5′-diphosphate (GTP), and adenosine deaminsane were purchased from Sigma-Aldrich (St. Louis, MO, USA). Quinpirole hydrochloride was purchased from Tocris Cookson (Ellisville, MO, USA). [^{35}S]GTPγS (1250 Ci/mmol) was bought from NEN-Perkin Elmer, Life Science (Boston, MA, USA). For acute cocaine administration, rats received a single intraperitoneal injection of saline (1 mL/kg) or cocaine (20 mg/kg in 0.9% saline) 1 week after arrival. This cocaine dose has been reported to induce locomotor behaviors and represents a middle range of behavioral responses in both sexes.[12]

Brain Dissections and *in vitro* [^{35}S]GTPγS Autoradiography

Forty minutes after their injection, animals were decapitated (following a 20-second exposure to CO_2). Brains were then removed, flash frozen in 2-methylbutane (−40°C), and stored at −80°C until used. Coronal sections (16 μm) from rostral frontal cortex and medial prefrontal cortex (R-FC and mPFC, respectively; 4.7 to 3.2 mm anterior to bregma), as well as caudate-putamen and and nucleus accumbens (CPu and NAc, respectively; 2.2 to 0.7mm anterior to bregma), were thaw-mounted onto Superfrost Plus Gold glass slides. Sections were then stored at −80°C until used. For [^{35}S]GTPγS autoradiography, quinpirole, a D_2-like receptor agonist, was used to induce G-protein activation as previously described[6,10]. Briefly, slides were preincubated in TME buffer (50 mM Tris-HCl, 3 mM $MgCl_2$, 0.2 mM EGTA and 100 NaCl, pH 7.4) for 10 minutes and transferred to assay buffer (TME buffer, 2 mM GDP, 10 mU/mL adenosine deaminase) for 15 minutes at room temperature. To determine agonist-stimulated [^{35}S]GTPγS binding, slides were incubated in assay buffer containing 0.1 mM [^{35}S]GTPγS (1250 Ci/mmol), DTT (0.2 mM), and quinpirole (100 uM) for 2 hours at room temperature. Basal [^{35}S]GTPγS and non-specific binding were determined by incubating slides without agonist and in the presence of 10 μM unlabeled GTPγS, respectively. Slides were then washed twice for 3 minutes in ice-cold 50 mM Tris-HCl (pH 7.4) and briefly dipped into ice-cold deionized water. Slides were dried overnight and then exposed to Hyperfilm (Amersham Bioscience, UK) for 24 hours.

Statistics

The quinpirole-stimulated G-protein activity was calculated by subtracting the optical density (OD) of basal binding from that of agonist-stimulated binding. Data were expressed as relative stimulation ratio [stimulation ratio = (stimulated OD – basal OD)/ basal OD]. Within each sex group, *t* test was used for analyzing specific areas. A two-way ANOVA (treatment × sex) followed by Bonferroni post hoc test was used to further determine sex differences. For all analyses, P value ≤.05 was significant.

RESULTS

In both male and female rats, quinpirole stimulated [^{35}S]GTPγS binding in all brain regions studied—including the R-FC, mPFC, CPu, and NAc—as compared with basal controls (Figure 1). Only in the mPFC did cocaine-treated females exhibit higher [^{35}S]GTPγS binding than their saline-treated controls ($t(6) = 2.48$, $P < .05$; Figure 2A). A significant interaction of

treatment and gender was also observed in the mPFC [F (1, 13) = 0.63, P=.025]; saline-treated male rats had higher [^{35}S]GTPγS binding than their female counterparts (P<.05; Figure 2C). No changes were observed in the NAc or CPu (Figure 2B, C).

DISCUSSION

Acute cocaine administration has been shown to increase dopamine levels in the mPFC, an area that receives dopaminergic inputs arising from the ventral tagmental area (VTA) and is rich in dopamine D_2-like receptors.[13] Activation of dopamine D_2-like receptors in the mPFC has been postulated to inhibit not only cocaine-induced locomotor augmentation, initiation, and expression of behavioral sensitization but also dopamine overflow in the NAc.[14] Because females have augmented locomotor response and increased behavioral sensitization to cocaine, the higher dopamine D_2 receptor-stimulated G-protein may represent a compensatory mechanism in reaction to augmented dopamine overflow and/or dopaminergic system activation induced by cocaine stimulation.

In this study, male control rats exhibited higher [^{35}S]GTPγS binding in the mPFC than did females, suggesting a higher tonic dopamine D_2-like receptor activation and thereby gating of the excitatory outflow to the striatum. Thus, this finding suggests a sexually dimorphic pattern in G-protein activation before cocaine exposure. Gonadal hormones have been found to regulate the D_2 receptors.[15] Additionally, estrogen treatment in ovariectomized rats induces a decrease in dopamine D_2-like receptor-mediated [^{35}S]GTPγS binding in the VTA and increases behavioral responses after acute cocaine.[4] Thus, the hormonal profile of females may underlie the sex difference observed in [^{35}S]GTPγS binding in the mPFC.

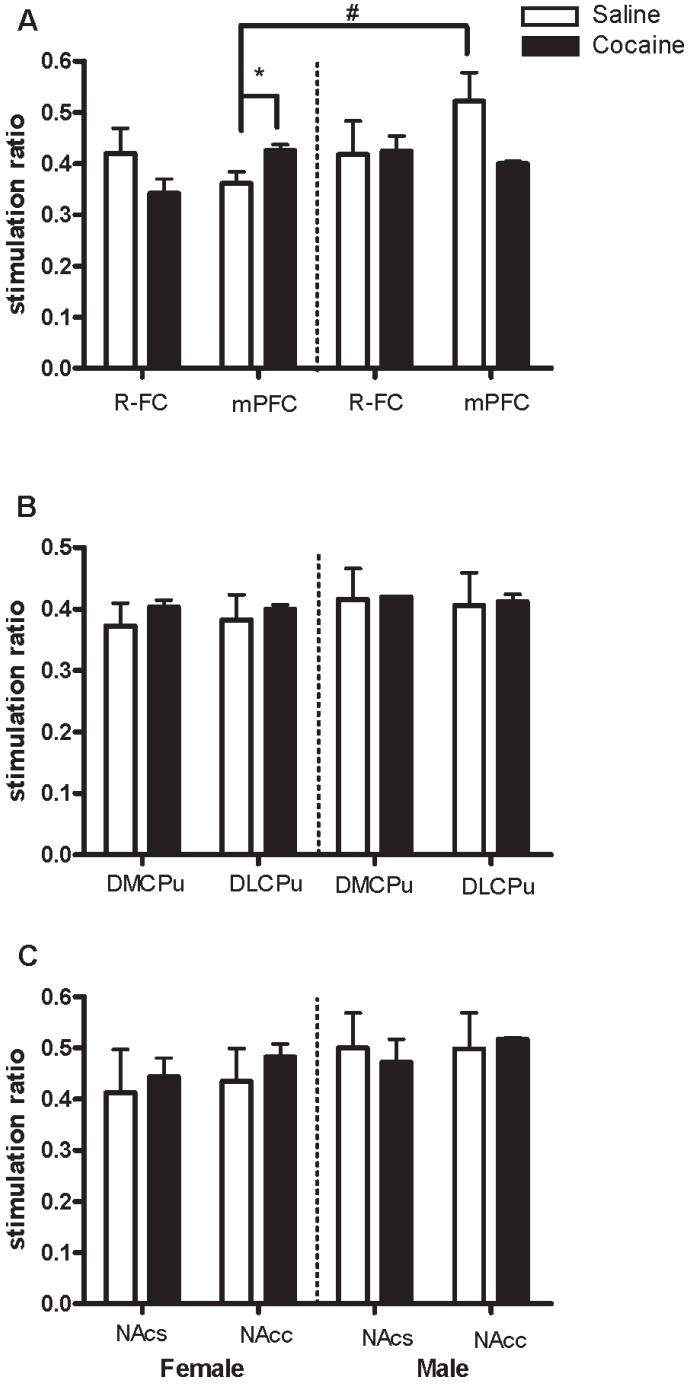

Fig 2. Mean + SEM for D_2-like agonist stimulated G-protein activation in: (A) rostral frontal cortex and medial prefrontal cortex (R-FC and mPFC); (B) dorsal medial and dorsal lateral CPu (DM and DL CPu); (C) core and shell of NAc (NAcc and NAcs) of male and female rats after saline or cocaine injections. * P<.01 compared with cocaine-treated rats; # P<.01 compared with male rats

The mPFC is a part of the brain reward circuitry implicated in the formation of learning and memory.[16] For example, lesions of the mPFC have been shown to block the development and reinstatement of cocaine-induced conditioned place preference (CPP) and cocaine self-administration, whereas di-

rect intra-mPFC cocaine infusion reinstates the cocaine-seeking behavior.[17] Sex differences also exist in the reinforcing and rewarding properties of cocaine: female rats demonstrate a rapid acquisition of cocaine self-administration and have a greater response during the reinstatement phase than do male rats.[18] Compared with males, female rats develop CPP at lower doses of cocaine and with fewer conditioning days.[14] Since the activation of dopamine D2-like receptors in the mPFC antagonizes the acute and chronic behavioral effects of cocaine,[14] it is reasonable to postulate that the lower mPFC physiological [^{35}S]GTPγS binding levels observed in female rats may contribute to their higher reinforcing and rewarding response to cocaine.

After acute cocaine administration, the D_2-like receptor-mediated G-protein activation was not significantly altered in the NAc or CPu. This finding is in line with previous studies demonstrating that chronic, but not acute, cocaine administration alters pertussis toxin-sensitive G-protein (Gαi/o) in the NAc.[5,19] Recently, our laboratory reported that although a rapid down-regulation of striatal D_1 receptor binding sites after acute cocaine was observed in males, it did not occur in D_2 receptor binding levels.[12] Thus, in the CPu and/or NAc, the dopamine D_2-like receptors may play a limited role in sexually dimorphic behavioral patterns in response to acute cocaine administration.

Implications for Improving Health Disparities

According to the National Household Survey on Drug Abuse, approximately 3.2 million men and 1.4 million women used cocaine at least once during 2000. For the past 3 years, cocaine has been the second most commonly found drug in the systems of persons treated in emergency rooms, and since 1999 drug-related ER visits involving females have increased 9% whereas the percentage involving males has not changed (www.nih.nida.gov). As more attention is paid to gender, it is becoming apparent that males and females react differently to administration of cocaine. It is well established that females and males have different dopaminergic mediated intracellular responses, and this difference may underlie the higher rewarding sensitivity and psychomotor responses in females. This article provides further evidence of sex differences in dopaminergic mediated intracellular responses to cocaine. The impact of sex differences in the dopaminergic responses to cocaine needs further studies.

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