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Influence of progesterone on GAD_{65} and GAD_{67} mRNA expression in the dorsolateral striatum and prefrontal cortex of female rats repeatedly treated with cocaine

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Abstract

Female rats are intensely affected by cocaine, with estrogen probably playing an important role in this effect. Progesterone modulates the GABA system and attenuates the effects of cocaine; however, there is no information about its relevance in changing GABA synthesis pathways after cocaine administration to female rats. Our objective was to investigate the influence of progesterone on the effects of repeated cocaine administration on the isoenzymes of glutamic acid decarboxylase (GAD_{65} and GAD_{67}) mRNA in brain areas involved in the addiction circuitry. Ovariectomized, intact and progesterone replacement-treated female rats received saline or cocaine (30 mg/kg, *ip*) acutely or repeatedly. GAD isoenzyme mRNA levels were determined in the dorsolateral striatum (dSTR) and prefrontal cortex (PFC) by RT-PCR, showing that repeated, but not acute, cocaine decreased GADs/β-actin mRNA ratio in the dSTR irrespective of the hormonal condition (GAD_{65}: $P < 0.001$; and GAD_{67}: $P = 0.004$). In the PFC, repeated cocaine decreased GAD_{65} and increased GAD_{67} mRNA ratio ($P < 0.05$). Progesterone replacement decreased both GAD isoenzymes mRNA ratio after acute cocaine in the PFC ($P < 0.001$) and repeated cocaine treatment reversed this decrease ($P < 0.001$). These results suggest that cocaine does not immediately affect GAD mRNA expression, while repeated cocaine decreases both GAD_{65} and GAD_{67} mRNA in the dSTR of female rats, independently of their hormonal conditions. In the PFC, repeated cocaine increases the expression of GAD isoenzymes, which were decreased due to progesterone replacement.

Key words: Cocaine; Progesterone; GAD mRNA; Dorsolateral striatum; Prefrontal cortex

Introduction

Chronic intermittent administration of psychostimulants produces sensitization that is relevant to addictive behaviors (1). Although drugs of abuse possess diverse neuropharmacological profiles, activation of the mesocorticolimbic system constitutes a common pathway by which various drugs of abuse mediate their acute reinforcing effects. The long-term neuroadaptations in this circuitry underlie the transition to drug dependence and cycles of relapse (2).

The striatum is a distinct region in the brain striatal complex that is a putative site of action for the motor activating effects of drugs of abuse and associated neuroadaptations and may mediate facets of cocaine reinforcement (1). Although the ventral striatum, or nucleus accumbens (NAc), has been extensively studied regarding its involvement in the reward effect of drugs of abuse, the dorsal striatum, or caudate-putamen, is directly implicated in motor habits, stimulus-response associations and habit learning (3). Cocaine exposure causes regionally specific effects on neural processing in the striatum and these effects may promote the habituation of behavior by a lasting shift in the balance of associative encoding away from the ventral striatum and toward the dorsal striatum (3).

The prefrontal cortex (PFC) also has an important role in the development of addictive behaviors and may contribute to the development of psychostimulant-induced reward and addiction (4). In both areas, dopaminergic and glutamatergic projections are recognized as structures that are central to the behavioral responses to cocaine (5). The glutamatergic and dopaminergic excitatory influences are opposed by the GABAergic inhibitory

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system, which also appears to be altered by psychostimulants (6). The striatum contains primarily medium-sized GABAergic spiny projection neurons. In fact, its vast majority of neurons (90-95%) contain GABA (7). In the PFC, GABAergic neurons compose 25 to 35% of all neurons (8) and may reduce neuronal excitability and cortical functions output (9).

In addition to influencing the glutamate and dopamine systems and indirectly modifying GABA signaling, psychostimulants may also directly influence the activity of GABAergic neurons. Chronic cocaine administration has been shown to both increase and decrease GABA turnover in different brain areas (10,11), to decrease the release of GABA in the ventral tegmental area (12), and to increase extracellular GABA in the PFC (13). GABA is synthesized by two isoenzymes of glutamic acid decarboxylase, GAD_{65} and GAD_{67} (14), which differ in their contributions to the synaptic and non-synaptic pools of GABA (15,16). Repeatedly cocaine-treated animals show variable and apparently contradictory changes in the protein levels of GAD_{65} and GAD_{67} isoenzymes in different brain areas of male rats. Increased GAD_{65} has been detected in the anterior hypothalamus and amygdala, while decreased GAD_{65} has been reported in the lateral septum (14) and no changes in GAD_{65} have been observed in the ventral pallidum (17), NAc and caudate (18). Repeated psychostimulant use increased GAD_{67} mRNA in the central amygdala (19), decreased GAD_{67} mRNA (20) and GAD_{67} protein has been detected after repeated cocaine in the NAc (18) and no changes in the GAD_{67} mRNA levels were observed in the medial PFC, dorsolateral striatum (dSTR) and NAc (21).

The hormonal differences between males and females may influence cocaine use and its effects, with females experiencing more intense acute behavioral effects than males (22,23). Female rats also show greater responses to repeated cocaine administration such as sensitization, conditioned place preference, self-administration, and dose escalation (24). Gonadal hormones may play an important role in these gender differences (25). Although the data support the notion that estrogen is predominantly responsible for the differences between males and females in response to cocaine use, it appears that this hormone may not be the only influence. While estrogen induces an increase in locomotor activity and in cocaine self-administration behavior (24,26), progesterone seems to attenuate these cocaine-mediated effects (27,28). In female rats, exogenous treatment with progesterone reverses the effects of estrogen on the acquisition of self-administration of cocaine (29), attenuates the motor responses to cocaine (30,31) and inhibits the place preference of cocaine (28). Additionally, cocaine-induced hyperactivity and self-administration are the lowest in the phase of the estrous cycle when progesterone peaks (27,29).

In view of the possible participation of the GABA system in the neurobiology of the plastic changes that take place after repeated cocaine use, to which females are more sensitive, it is important to note that female hormones also modulate the GABAergic system by regulating the expression of the neurotransmitter synthesizing enzymes or its receptors. Progesterone and its metabolite allopregnanolone act as positive modulators of $GABA_A$ receptors (32). Recent evidence suggests that the mechanism by which neurosteroids modulate GABAergic function is through alteration of the kinetics of the receptor (33). Studies have indicated that estradiol increases GAD expression in the hypothalamus and hippocampus of rats (34,35), whereas estradiol plus progesterone treatment reverses it in the preoptic area and hippocampus (36). However, even though the GAD content was increased in the olfactory bulb of male rats by individual administration of estradiol and progestins, progestins attenuated the effects of estradiol on GAD in this system (37). Although the interference of progesterone with the GABA system may be expected to affect cocaine treatment, particularly in females, no information exists on the relevance of the changes in GAD plasticity due to repeated cocaine administration in female rats and the influence of progesterone on such changes. Therefore, the objective of the present study was to determine whether female hormones modulate the changes in the GABA system due to the effects of cocaine administration by comparing intact and castrated females and investigating the influence of progesterone replacement on the effects of cocaine on GAD_{65} and GAD_{67} mRNA expression in the PFC and dSTR of female rats.

Material and Methods

Animals and experimental design

Female Wistar rats weighing 250-350 g (N = 70) were obtained from the Animal House of Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA). Rats were housed with free access to food and water and were maintained in a temperature-controlled room (22 ± 2°C) under a 12-h light/dark cycle with lights on from 7:00 am to 7:00 pm. Bilateral ovariectomy was performed on 50 rats 2 weeks before starting the drug administration protocol under anesthesia with intraperitoneal (*ip*) xylazine (10 mg/kg) and ketamine hydrochloride (75 mg/kg). The rats submitted to ovariectomy were randomly assigned to the progesterone (PRO, N = 20) or ovariectomized (OVX, N = 30) group. An additional group of 20 intact female rats (INT) was included. Rats of the PRO group received subcutaneous (*sc*) progesterone, 1 mg/kg, N = 20, while OVX and INT rats received sesame oil daily. On the first experimental day, vaginal secretion was collected from all rats to confirm that castration and progesterone replacement were effective and that the intact females were equally distributed in the different phases of the estrous cycle. All experiments followed the Guidelines of the International Council for Laboratory Animal Science (ICLAS) (38) and were approved by the Ethics Committee for Animal Experimentation of UFCSPA.

Drug administration protocol

Ten rats from each the INT, OVX and PRO groups were randomly assigned to acute (ACT) or repeated (RPT) cocaine treatment groups. ACT animals received 1 mL/kg saline *ip*

and the RPT rats received 30 mg/kg cocaine hydrochloride, *ip* (ENILA, Brazil) for 5 consecutive days and all animals of the both groups received a challenge with the same dose of cocaine *ip*. A control (CTR) group consisting of OVX rats was included in the study. This group, which received 1 mL/kg saline *ip* throughout the study period, was used as negative control for drug treatment and for the presence of female sexual hormones. In a pilot study performed in our laboratory, comprising up to 30 animals per hormonal condition, no difference in GAD isoenzyme mRNA was detected between INT, OVX and estrogen-treated (2.5 µg/kg, *sc*) or progesterone-treated (1 mg/kg, *sc*) OVX rats (Table 1).

Two weeks after ovariectomy, the animals received the first oil or progesterone injection according to their assigned group 24 h before the beginning of the procedures. Progesterone or oil was injected daily thereafter. On day 2, i.e., the first experimental day, rats from the CTR and ACT groups received saline and rats from the RPT group received cocaine. Over the next 5 consecutive days, the animals received either saline or cocaine once a day, as scheduled. Injections of saline/cocaine and oil/PRO were discontinued for 6 days. On day 13, CTR animals received saline *ip* and ACT and RPT animals received a challenge dose of cocaine. This protocol is similar to those used in behavioral sensitization of rats submitted to repeated treatment with cocaine. One hour after the cocaine challenge, the animals were decapitated, the dSTR and PFC were then dissected, immediately frozen in liquid nitrogen and stored at -80°C for further mRNA analysis. The dissection of these structures was performed by initially separating the brain hemispheres and removing the PFC from both hemispheres. To dissect the dSTR, the cortex was flipped mediolaterally to expose the medial surface of the striatum and the lateral limit of the striatum was carefully dissected along its perimeter.

RNA extraction and RT-PCR analysis

A semiquantitative RT-PCR technique was used to determine the levels of GAD_{65} and GAD_{67} mRNA. Total RNA was isolated from 70-90 mg dSTR and PFC using a Trizol® isolation reagent kit (Invitrogen™ Life Technologies Inc., USA) according to manufacturer instructions. Total RNA purity was assessed by UV spectrophotometry (Gen Quant II®, Amersham Pharmacia Biotech, Brazil). A 3-µg aliquot of total RNA was reverse transcribed using the SuperScript™ first-strand synthesis system for RT-PCR (Invitrogen™ Life Technologies Inc.), according to manufacturer instructions.

Relative RT-PCR was performed to measure gene expression of GAD_{65} and GAD_{67} mRNAs. Specific oligonucleotides derived from the coding region of the published sequences of GAD_{65} (5' GCTCTACGGAGACTCTGAGAAG 3' and 5' CGGTTGGTCTGACAATTCCC 3') and GAD_{67} (5' TGTGGCGTAGCCCATGGATG 3' and 5' ACTGGTGTGG GTGGTGGAAG 3') were used. Primer sequences were designed to span intron regions when genomic sequence data were available, resulting in the predicted 318- and 330-bp sequence, respectively. The β-actin primer set (5' TGTGATGGTGGGAATGGGTCAG 3' and 5' TTGATGTC ACGCACGATTTCC 3') that generated a 515-bp product was used as an internal control. β-actin was co-amplified within the same reaction to evaluate inter-sample variation in cDNA contents and PCR efficiency.

PCR included 2 µL of the RT product and was carried out with Taq DNA polymerase (Invitrogen™ Life Technologies Inc.) in a final volume of 25 µL, 1.5 mM $MgCl_2$, 0.2 µM of the specific primer, and 200 µM dNTPs. PCR was performed in a Mastercycler® Personal thermal cycler (Eppendorf, Germany). The amplification protocol consisted of an initial denaturation step at 94°C for 3 min, followed by 40 cycles at 94°C for 1 min, annealing at 60°C for 1 min, extension at 72°C for 1 min, and a final extension at 72°C for 5 min. The linear amplification range for each gene was tested on the adjusted cDNA. The less expressed transcripts of GAD_{65} and GAD_{67} required >32 PCR cycles for detection. β-actin primers were added when 5 cycles were remaining in the specified gene's linear amplification range. RT-PCR assays without cDNA samples were carried out as negative controls. All reactions were performed in duplicate. After amplification, 10 µL of the PCR products was analyzed on

Table 1. mRNA expression of GAD isoenzymes (GAD_{65} and GAD_{67}) in the prefrontal cortex and dorsolateral striatum of female rats under different hormonal conditions.

	Dorsolateral striatum		Prefrontal cortex	
	GAD_{65} (N = 83)	GAD_{67} (N = 86)	GAD_{65} (N = 73)	GAD_{67} (N = 76)
INT	1.59 ± 0.15 (25)	1.09 ± 0.10 (24)	0.75 ± 0.04 (28)	0.93 ± 0.03 (25)
OVX	1.37 ± 0.10 (26)	1.06 ± 0.09 (28)	0.85 ± 0.04 (24)	0.89 ± 0.03 (29)
OVX + P	1.52 ± 0.14 (17)	1.06 ± 0.11 (18)	0.64 ± 0.07 (11)	0.80 ± 0.05 (12)
OVX + E	1.61 ± 0.17 (15)	1.01 ± 0.09 (16)	0.75 ± 0.07 (10)	0.97 ± 0.05 (10)
	$F_{(3,77)}$ = 1.063; P = 0.370	$F_{(3,85)}$ = 0.137; P = 0.938	$F_{(3,67)}$ = 2.349; P = 0.08	$F_{(3,70)}$ = 2.192; P = 0.097

Data are reported as means ± SEM. INT = intact; OVX = ovariectomized; OVX + P = ovariectomized treated with 1 mg/kg progesterone, *sc;* OVX + E = ovariectomized treated with 2.5 µg/kg estrogen, *sc*.

a 1.5% ethidium bromide agarose gel. The intensity of each band was assessed by optical densitometry (ImageMaster® VDS, Amersham Pharmacia Biotech). DNA band intensity was normalized against the corresponding values of the β-actin band intensity. Data are reported as the ratio of the GAD_{65}/β-actin and GAD_{67}/β-actin and these were used for statistical analysis.

Statistical analysis

Analysis of the mRNA optical density data was performed via a three-way ANOVA, considering the GAD isoenzyme measured (GAD_{65} and GAD_{67}), treatment with cocaine (CTR, ACT, and RPT) and hormonal condition (INT, OVX, and PRO) in each brain region. Because there was a significant difference between the amount of mRNA of isoenzymes, two-way ANOVA was performed for each cerebral structure to analyze GAD_{65} or GAD_{67}, considering the drug treatment (CTR, ACT and RPT) and hormonal condition (INT, OVX and PRO). One-way ANOVA was used to compare ACT and RPT cocaine treatments for INT, OVX and PRO with the controls OVX. Two-way ANOVA was performed to compare the GAD isoenzyme mRNA expression (GAD_{65} and GAD_{67}) and brain regions (dSTR and PFC). The Student-Newman-Keuls test was used for *post hoc* comparisons when appropriate. Data are reported as means ± SEM. A P value of less than 0.05 was considered to be significant.

Results

The RT-PCR technique was concluded with 7-10 samples per group.

In general, a significant main effect of brain region was found ($F_{(1,281)}$ = 95.683; P < 0.001), with the dSTR (1.43 ± 0.04) showing higher GAD mRNA expression than the PFC (0.83 ± 0.04). Also, there was a significant difference ($F_{(1,281)}$ = 12.846; P < 0.001) in GAD isoenzyme mRNA expression, with GAD_{65} (1.24 ± 0.04) presenting higher mRNA expression than GAD_{67} (1.02 ± 0.04).

Furthermore, an interaction between brain regions and GAD isoenzymes was found ($F_{(1,281)}$ = 35.040; P < 0.001). In the PFC, GAD_{67} mRNA (0.90 ± 0.06) was expressed more than GAD_{65} mRNA (0.76 ± 0.06). In contrast, in the dSTR, the expression of GAD_{65} mRNA (1.72 ± 0.06) was higher than the expression of GAD_{67} mRNA (1.14 ± 0.06).

Prefrontal cortex

As shown in Figure 1A, in the PFC of OVX and INT rats, repeated cocaine decreased GAD_{65} mRNA expression compared to baseline levels (CTR animals; $F_{(3,28)}$ = 3.546, P = 0.027). In PRO rats, acute cocaine decreased GAD_{65} mRNA ($F_{(3,30)}$ = 4.463, P = 0.010) and repeated treatment reversed this decline. Additionally, rats in the ACT-PRO subgroup had lower mRNA expression of GAD_{65} than rats in the ACT-OVX and ACT-INT subgroups ($F_{(2,38)}$ = 3.267, P = 0.049).

As shown in Figure 1B, repeated cocaine increased GAD_{67} mRNA levels in the PFC of INT rats compared to baseline levels ($F_{(3,33)}$ = 7.012, P < 0.001) and to ACT-INT animals ($F_{(1,39)}$ = 4.562, P = 0.039). Also, in PRO rats, acute cocaine decreased GAD_{67} mRNA compared to baseline levels ($F_{(3,34)}$ = 7.711, P < 0.001), an effect reversed by repeated treatment. When analyzing the hormonal condition, ACT-PRO animals exhibited a lower expression of this isoenzyme compared to OVX and INT rats of the same drug treatment group. In the repeated group, an increase in GAD_{67} mRNA expression was observed in INT rats compared to OVX and PRO replacement rats ($F_{(2,39)}$ = 15.485, P < 0.001).

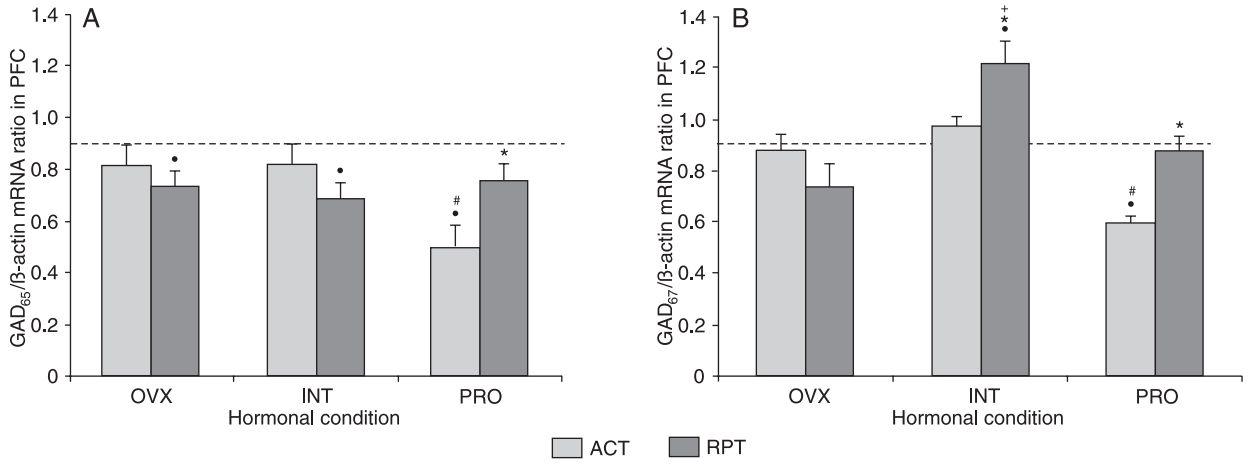

Figure 1. mRNA expression of glutamic acid decarboxylase isoenzymes (GAD_{65} and GAD_{67}) in the prefrontal cortex (PFC) of female rats according to their hormonal condition. Data are reported as means ± SEM. ACT = acute cocaine; CTR = control; INT = intact; OVX = ovariectomized; PRO = progesterone replacement; RPT = repeated cocaine. *Panel A*: •Statistically different from CTR-OVX (dashed line). *Statistically different from ACT-PRO. #Statistically different from ACT-INT and ACT-OVX. *Panel B*, •Statistically different from CTR-OVX (dashed line). *Statistically different from ACT. #Statistically different from ACT-OVX and ACT-INT. +Statistically different from RPT-OVX and RPT-PRO (two-way ANOVA and Student-Newman-Keuls *post hoc* test; P < 0.05).

Additionally, an interaction was observed between cocaine treatment and the hormonal condition of female rats ($F_{(2,39)}$ = 6.001, P = 0.005).

Dorsolateral striatum

As shown in Figure 2A, GAD_{65} mRNA expression in the dSTR was significantly decreased after repeated cocaine treatment compared to baseline levels (CTR) ($F_{(3,25)}$ = 9.197, P < 0.001) in relation to the acute group of intact and progesterone replacement animals ($F_{(1,35)}$ = 17.431, P < 0.001). Acute treatment decreased the GAD_{65} mRNA levels compared to baseline, but only in OVX rats ($F_{(3,28)}$ = 3.446, P = 0.030). Considering the hormonal condition of the rats, OVX rats subjected to acute treatment with cocaine showed a reduction in GAD_{65} mRNA expression compared to intact and progesterone replacement rats submitted to the same drug treatment ($F_{(2,35)}$ = 5.073, P = 0.012). In repeatedly treated animals, we observed a decrease in the expression of this mRNA in OVX rats compared to intact rats ($F_{(2,16)}$ = 3.941, P = 0.041). The GAD_{67} mRNA levels in the dSTR of female rats were significantly decreased under repeated treatment with cocaine when compared to baseline in all hormonal conditions ($F_{(3,38)}$ = 5.321, P = 0.004) and when compared to the acutely treated rats of the same hormonal group ($F_{(1,43)}$ = 14.944, P < 0.001). There were no differences concerning the hormonal condition of female rats (Figure 2B).

Representative agarose gels of the RT-PCR measurements of dSTR and PFC GAD_{65} mRNA and GAD_{67} mRNA are shown in Figure 3.

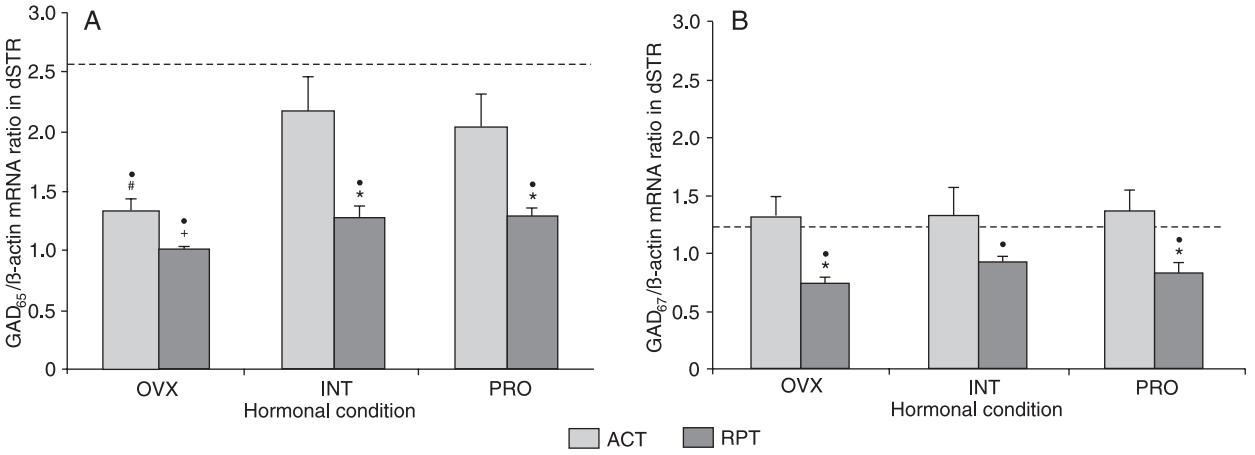

Figure 2. mRNA expression of GAD isoenzymes (GAD_{65} and GAD_{67}) in the dorsolateral striatum (dSTR) of female rats, according to the hormonal condition. Data are reported as means ± SEM. ACT = acute cocaine; CTR = control; INT = intact; OVX = ovariectomized; PRO = progesterone replacement; RPT = repeated cocaine. *Panel A*: •Statistically different from CTR-OVX (dashed line). *Statistically different from ACT. #Statistically different from ACT-INT and ACT-PRO. +Statistically different from RPT-INT. *Panel B*: •Statistically different from CTR-OVX (dashed line). *Statistically different from ACT. (two-way ANOVA and Student-Newman-Keuls *post hoc* test; P < 0.05).

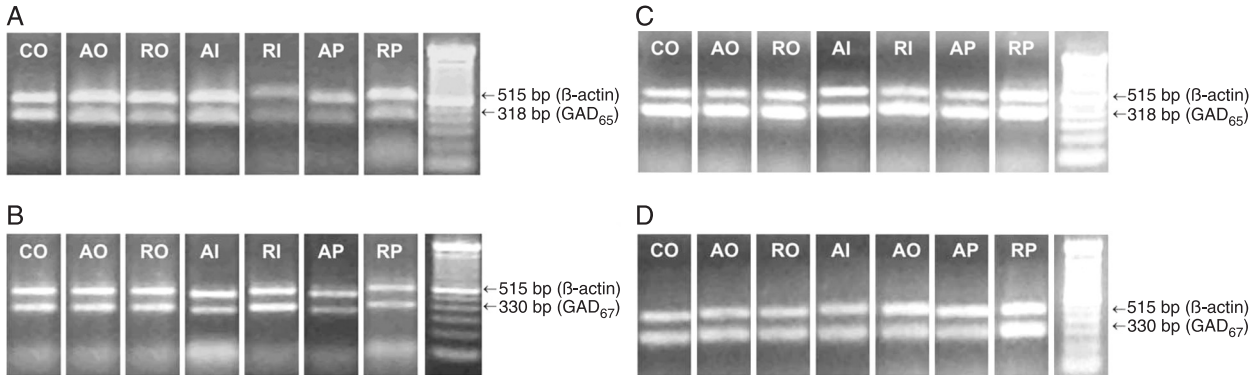

Figure 3. Ethidium bromide-stained agarose gels showing RT-PCR products amplified from mRNA: *A*, From the prefrontal cortex showing GAD_{65} and β-actin bands; *B*, From the prefrontal cortex showing GAD_{67} and β-actin bands; *C*, From the dorsolateral striatum showing GAD_{65} and β-actin bands; D, From the dorsolateral striatum showing GAD_{67} and β-actin bands. Abbreviations: control (C), acute cocaine (A), repeated cocaine (R), ovariectomized (O), intact (I), progesterone replacement (P).

Discussion

In this study, we show that female hormones, mainly progesterone, modulate the cocaine-induced changes in the GABA synthesis apparatus in the PFC. This report documents some genomic changes in GABA synthesis enzymes in different brain areas in response to the prolonged use of cocaine. Our results are relevant because GABA is the inhibitory neurotransmitter naturally counterbalancing glutamate and dopamine increases due to cocaine abuse.

We also show that different brain areas are associated with different effects of cocaine and hormonal interactions on the amount of mRNA of the GAD isoenzymes. Independently of the hormonal conditions of female rats (absence or presence of hormonal cycling or progesterone replacement), repeated (but not acute) cocaine decreased both GAD_{65} and GAD_{67} gene expression in the dSTR. In the PFC, female hormones modulated the effect of cocaine treatment, depending on the hormones and enzymes being studied. In short, castrated or intact female rats showed decreased GAD_{65} mRNA in the PFC when repeatedly treated with cocaine, repeated treatment with cocaine increased GAD_{67} mRNA in intact females, but not in castrated ones, and repeated cocaine reversed the decrease in GAD_{65} and GAD_{67} mRNA seen after progesterone replacement.

Previous studies had only used male rats (14,17-21), while the effects of cocaine on the genomic apparatus of GAD isoenzymes in females are reported here for the first time. Our results confirm that GAD mRNA changes are a general phenomenon after prolonged cocaine administration, appearing also in females, even though there are differences compared to males. Intact male rats that were repeatedly treated with psychostimulants showed neither a change in GAD_{65} levels in the ventral pallidum (17), NAc and caudate (18), nor in GAD_{67} mRNA levels in the PFC or striatum (21). In contrast, intact male rats showed increased GAD_{65} in the hypothalamus and amygdala, decreased GAD_{65} in the lateral septum (14), decreased GAD_{67} in the NAc (18,20), and increased GAD_{67} mRNA in the amygdala (19). In the present study, intact females showed decreased GAD_{65} mRNA and increased GAD_{67} mRNA expression in the PFC after repeated cocaine administration, while in the dSTR there was a decrease in both GAD isoenzyme mRNAs in the rats that were repeatedly treated with cocaine. Therefore, one clearly sees that there are gender differences regarding the effects of cocaine on the GABA synthesis enzyme apparatus. It remains to be established if the effects of repeated cocaine administration on GAD isoenzyme synthesis control occur in the other brain areas of female rats and if the alterations in GAD mRNA are maintained for long periods of time as described for males (19).

Our results with female rats confirmed data obtained in other studies demonstrating that acute cocaine treatment does not induce major changes in either GAD isoenzyme mRNA expression. In fact, the literature points out that at least 4 to 6 h are necessary to observe changes in GAD mRNA induced by acute administration of psychostimulants (20,21). The time interval between cocaine intake and changes in GAD expression may indicate neuroadaptation that occurs in the GABA system in response to cocaine.

In the PFC of castrated rats, progesterone replacement treatment reduced, by itself, both isoenzymes and repeated cocaine reversed this effect, increasing the mRNA expression of both GAD isoenzymes. It may be conjectured that progesterone induces neuroplastic changes in the GABA system due to its positive modulatory effect on $GABA_A$ receptors (32), which could induce a negative feedback in presynaptic neurons and a consequent tentative decrease in GABA synthesis (20). Interestingly, it seems that the PFC is more sensitive to these effects or that, conversely, the dSTR is resistant to the effects of progesterone. Future studies should clarify this question. Overall, these results suggest that there is an interaction between cocaine and progesterone in the PFC.

The PFC may be one of the areas showing the main interactions between female hormones and cocaine use. Cocaine induced a decrease in GAD_{65} mRNA in both castrated and intact females. Therefore, there was no important influence of the natural hormonal secretion on the effects of cocaine on GAD_{65}. On the other hand, intact females presented higher GAD_{67} levels after repeated cocaine administration. Hormones influenced the effects of cocaine because OVX rats did not show the same effect. It is possible that the normal intact cycling females showed this increase in GAD_{67} mRNA after repeated cocaine due to the influence of progesterone because the profile of the effect on intact rats was similar to that seen in the OVX females receiving progesterone replacement.

Castrated female rats are more similar to male rats with respect to the lack of changes in GAD_{67} levels in the PFC (21), while they maintain the female profile of GAD_{65} decrease in the dSTR compared to the lack of a change that is seen in male rats (18). The results with castrated females corroborated the expected fundamental differences between males and females. Thus, all gender differences depend on the actual presence of female hormones.

The enhancement in GAD_{67} mRNA expression in the PFC of intact female rats may indicate the higher need for synthesis of cytoplasmic GABA and consequently a greater mobilization of the inhibitory system in this brain region. This mobilization is probably required by the exacerbation of the effects of repeatedly administered cocaine in females with circulating hormones, which has been widely reported in the literature (27,39).

While GAD_{65} is highly enriched in the nerve terminals and is believed to regulate vesicular GABA synthesis, GAD_{67} is found throughout the GABAergic neurons and may be involved in the synthesis of cytosolic or "metabolic" GABA (16). Cocaine treatment has been reported to release GABA into the extracellular space. The reduction of GAD_{65} and GAD_{67}

mRNA in the dSTR of our rats may have been associated with an extensive presynaptic GABA release maintained by repeated cocaine (40). Extensive GABA release may lead to a subsequent decrease in synthesis of vesicular and cytoplasmic GABA in the striatum (20) and in vesicular GABA in the PFC of castrated females by end-product inhibition or by the bombardment of synaptic receptors (15). Since GAD is expressed only in GABA neurons, it has served as a marker for GABAergic function (15). The observed shifts in the GABA synthesis pathway may be connected to the potentiation of the excitatory effects of psychostimulants, a consequence of long-term reduction of the inhibitory capacity of the central nervous system (20). In fact, cocaine induces a decrease in extracellular GABA in animals that had already self-administered cocaine (40). It seems that these neuroplastic changes may be connected to enhanced cocaine effects and dependence. Therefore, repeated cocaine administration may reduce the gene expression necessary for the synthesis of vesicular GABA in both the dSTR and PFC, while it may increase the gene expression needed for the synthesis of cytoplasmic GABA in the PFC and decrease it in the dSTR. One would like to know if these genomic effects are related to actual changes in the induction of GAD protein synthesis.

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