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Ritanserin, a 5HT2/1C Receptor Antagonist, Does Not Block Cocaine-Induced Behavioral Alterations and zif268 mRNA Expression in the Striatum of the Rats

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Cocaine induces immediate early gene expression and behavioral changes by blocking dopamine transporters in the terminals of nigrostriatal neurons in the striatum. The pharmacological role of serotonin 2/1C (5HT2/1C) receptors in cocaine-induced expression of zif268 (NGFI-A, egr1 and Krox-24) mRNA, a member of the zinc finger, was investigated using quantitative in situ hybridization histochemistry in vivo. Behavioral alterations induced by cocaine were also monitored in relation with blockade of the receptors. Systemic injection of ritanserin (1 mg/kg, s.c.), a 5HT2/1C receptor antagonist, did not reverse behavioral alterations and zif268 mRNA gene expression induced by 15 mg/kg cocaine, i.p., in the dorsal and ventral striatum. These data indicate that ritanserin-sensitive 5HT2/1C receptors are not necessary for cocaine-induced behavioral alterations and zif268 mRNA gene expression in the striatum.

Key Words: Cocaine, Ritanserin, 5HT2/1C receptors, In situ hybridization, zif268 mRNA, Behavioral alterations, Striatum

INTRODUCTION

Cocaine inhibits the presynaptic uptake of dopamine and serotonin (5-hydroxytryptamine, 5HT) (Bhat & Baraban, 1993). The inhibition of monoamine uptake on the 5HT system suggests that cocaine functions as an indirect 5HT agonist and produces behavioral alterations through 5HT receptor subtypes in the central nervous system (Cooper et al, 1995). Cocaine as a blocker of presynaptic transporters contributes to induce immediate early gene expressions, such as zif/268 and c-fos mRNAs (Daunais & McGinty, 1994, 1995, 1996; Moratalla et al, 1994), which is regulated by complicated intracellular signaling mechanisms.

Zif/268 is a member of the zinc-finger protein family (Lau & Nathans, 1987) which has been demonstrated to mediate stimulus-induced long-term adaptive changes in cellular physiology by binding on a guanine/cytosine-rich sites very similar to the SP1 site of regulatory regions of target genes (Rhodes & Klug, 1993). Zif268 mRNA is expressed at a relatively high basal level that is directly correlated with the level of neuronal activity (Christy et al, 1988; Worley et al, 1991). Following cocaine exposure, an increase in zif/268 mRNA is mainly expressed in neurons of both striosome and matrix compartments of rostral caudate/putamen, and it can be blocked by pretreatment with a D1 dopamine receptor antagonist in the striatum (Moratalla et al, 1992) and in the forebrain (Ennulat et al, 1994) of rats.

In the present study, it was hypothesized that the behavioral alterations and zif268 mRNA gene expression induced by cocaine are mediated by 5HT2/1C receptors in the striatum. This hypothesis was investigated in vivo by infusing ritanserin (RIT), a 5HT2/1C receptor antagonist, prior to cocaine using quantitative in situ hybridization histochemistry.

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METHODS

Animals

Adult male Wistar rats (250~270 g) were obtained from Charles River Laboratories, Raleigh, NC. Rats were individually housed during all experimental treatment in the Department of Comparative Medicine, East Carolina University School of Medicine. Food and water were provided ad libitum and the rats were maintained on a 12 hr light/dark cycle. All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques. All animal use procedures were approved by the University Animal Care and Use Committee and were accomplished in accordance with the provisions of the NIH "Guide for the Care and Use of Laboratory Animals".

Experimental design

Rats were randomly divided into four groups: vehicle (6.0 ml sterile dH_2O including 3 ml acetic acid, pH 3.5~4.0)+saline, n=5; saline+15 mg/kg cocaine HCl (NIDA, calculated as the salt, diluted in sterile saline, pH 7.4), n=5; 1 mg/kg RIT (RBI)+vehicle, n=5; 1 mg/kg RIT+15 mg/kg cocaine, n=5. The first solutions were injected subcutaneously (s.c.) and the second solutions were injected intraperitoneally (i.p.) 30 min after the first injection. Rats were observed and behaviorally rated 5 min before the first injection and every 5 min for 55 min after the second injection using a modified nine-point rating scale from Ellinwood & Balster, 1974: 1) asleep, inactive, 2) normal activities, grooming, 3) increased activity, 4) hyperactive running with jerky movements, 5) slow patterned repetitive exploration, 6) fast patterned repetitive exploration with hyperactivity, 7) stereotypy (repetitive sniffing/rearing in one location to the exclusion of other activities), 8) continuous gnawing, sniffing or licking 9) dyskinesia, seizure. The trained raters were blind to the drug treatments. The rats were anesthetized with Equithesin (5 ml/kg, i.p.) 55 min after the second injection and decapitated. The brains were removed from the skull and immediately frozen in isopentane at 40°C until they were sectioned for in situ hybridization histochemistry.

In situ hybridization histochemistry

Quantitative in situ hybridization histochemistry measuring zif/268 mRNA was performed as described (Wang & McGinty, 1997). Briefly, 12 mm sections throughout the striatum were cut in a cryostat and thaw-mounted onto gelatin/chromalum-coated slides. The sections were fixed, defatted and pretreated before hybridization using a synthetic cDNA oligonucleotide probe of 40 base pairs directed against zif/268 (Oncogene Research Products). The probe was labeled at the 3' end using α-^{35}S deoxyadenosine triphosphate (1075 Ci/mmol, New England Nuclear) and terminal deoxynucleotidal transferase (Boehringer Mannheim) to a specific activity of $7\sim12\times10^5$ cpm/μl. Pretreated slides were incubated with 1×10^6 cpm/μl/25 μl hybridization buffer/section overnight (20 hr) at 37°C in a humid environment. The slides were washed, dehydrated and exposed, along with ^{14}C standards (American Radiolabeled Chemicals), to Kodak X-OMAT film for 10 days. After film development, the slides were dipped in emulsion (Kodak NTB-3, IBI), exposed for 3 weeks, then developed with Dektol and fixed with Kodak fixer. Selected slides were lightly counterstained for Nissl substance with 0.1% Thionin.

Quantitation of the mRNA hybridization signals on X-ray films was performed using NIH Image software. The ^{14}C standards were measured, plotted against known dpm/mg, and converted to ^{35}S equivalents to

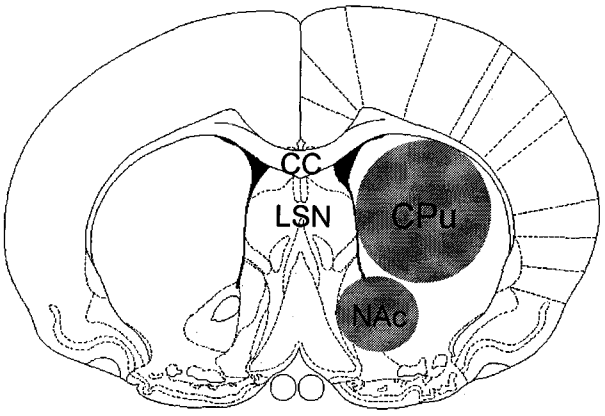

Fig. 1. Diagram illustrating areas measured by imaging on a coronal section of rat forebrain. Circled regions (CPu and NAc) indicate the areas in which hybridization signal of zif268 was measured by quantitative image analysis. CPu, Caudate-Putamen; NAc, Nucleus Accumbens; CC, Corpus Callosum; LSN, Lateral Septal Nuclei.

generate a calibration curve. After correcting for nonuniform illumination and subtracting film background by density slicing, the hybridization signals in the CPu were measured in each animal using a circular field with constant size of 130×130 pixels (Fig. 1). The signals in the NAc was also measured using a circular field including core area of NAc (diameter 45 pixels) (Fig. 1). All measurements were made at a point corresponding to 1.2 mm rostral to Bregma (Swanson, 1992) from the right three adjacent coronal sections of each animal matched across animals for their rostral/caudal level. Quantitative changes were expressed as the number of labeled pixels per area (area), the mean density of tissue in dpm/mg, and the integrated density which is the product of area times mean density (Wang & McGinty, 1997)

Statistics

Behavioral data were analyzed by calculating the area under the curve (AUC) for the rating values plotted against time. One-way ANOVA followed by a Tukey's HSD (honest significant difference) test in SAS (Cary, NC) was performed on the AUC values. Statistical significance in area, mean density, and integrated density between groups was determined by a nested two-way ANOVA followed by a Bonferroni-Dunn comparison of groups. This test is more precise when comparing least square means than Tukey's HSD test. All data except AUC values were subjected to a log transformation in order to obtain homogeneity of data.

RESULTS

Behavioral alterations after drug injections

Rats treated with vehicle/saline showed a minimal fluctuation of behavioral activity after injection. Rats treated with vehicle/cocaine displayed increased behavioral alterations within 10 min after the cocaine injection, and then gradually decreased their behavioral alterations along with the time course of the experiment. Quantitative analysis of the behavioral ratings (Fig. 2A) using overall AUC (Fig. 2B) revealed that RIT/saline did not show statistical significance in generating behavioral alterations, whereas vehicle/cocaine significantly altered behavior as compared with the vehicle/saline control group. Pretreatment of RIT did not block increase in behavioral changes induced by cocaine.

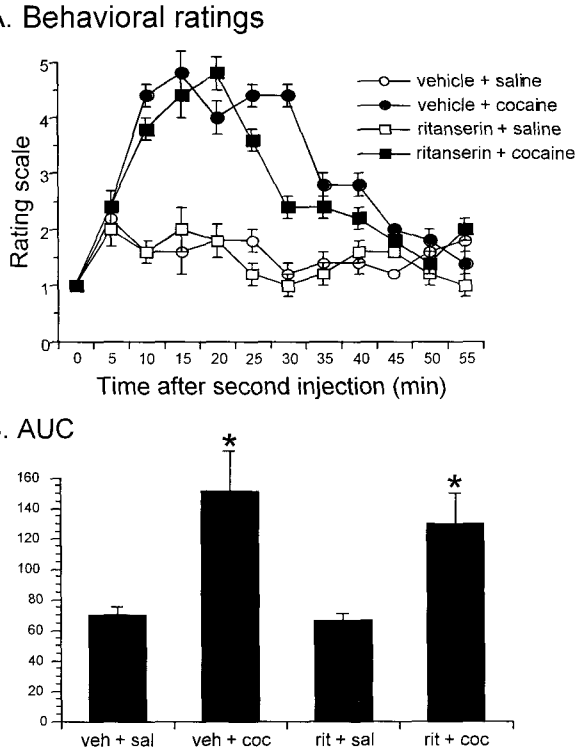

Fig. 2. Effects of ritanserin (1 mg/kg, s.c.) on the behavioral alterations induced by cocaine (15 mg/kg, i.p.) in the rat (A). Behavioral alterations were measured 5 min before the first injection and every 5 min for 55 min after the second injection. The AUC values represented in B demonstrate that ritanserin did not block cocaine-induced behavior. Veh, vehicle; sal, saline; coc, cocaine; rit, ritanserin. *$p < 0.05$ as compared with vehicle plus saline control group.

The effect of ritanserin on cocaine-induced zif/268 mRNA expression

Quantitation of zif268 mRNA expression in the CPu and NAc is illustrated in Fig. 3. In the CPu (Fig. 3A), RIT/saline group did not show significant increase in zif268 mRNA expression as compared with the vehicle/saline control group. Pretreatment of RIT did not block cocaine-induced zif268 mRNA expression. In the NAc (Fig. 3B), RIT/saline group did not alter zif268 mRNA expression as compared with the vehicle/saline control group. Pretreatment of RIT did not block cocaine-induced zif268 mRNA

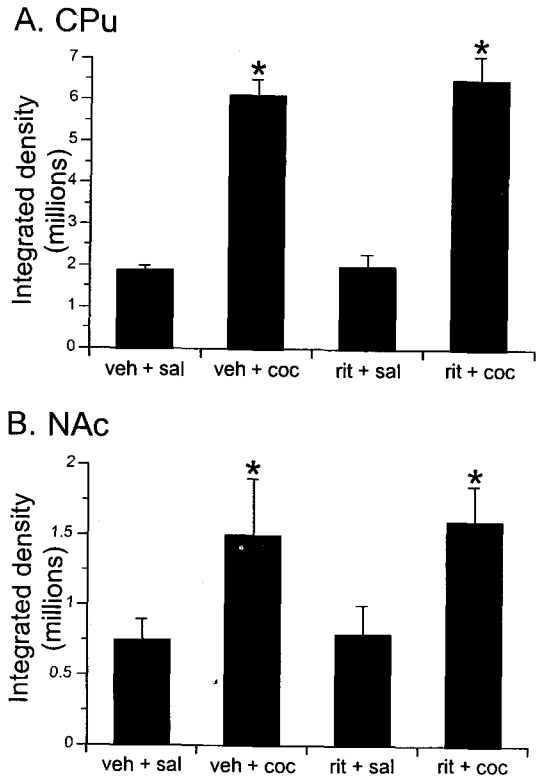

Fig. 3. Quantitative image analysis of the effects of ritanserin (1 mg/kg, s.c.) on the integrated density values for cocaine (15 mg/kg, i.p.)-induced zif268 mRNA expression in the CPu (A) and NAc (B) as illustrated in Fig. 1. Note that ritanserin did not block cocaine-induced zif268 mRNA expression in the two regions. Veh, vehicle; sal, saline; coc, cocaine; rit, ritanserin. *$p < 0.05$ as compared with vehicle plus saline control group.

expression as seen in the CPu.

DISCUSSION

In this study, RIT did not decrease behavioral alterations and zif268 mRNA expression induced by an acute injection of cocaine in the dorsal and ventral striatum. Investigation of the pharmacological effects of RIT revealed that behavioral changes and zif268 mRNA gene expression induced by cocaine are not closely related to 5HT2/1C receptors in the striatum.

The hypothesis that 5HT2/1C receptors would be involved in behavioral and immediate early gene expression was addressed by systemic administration of RIT and cocaine. The present data shows that RIT does not block cocaine-induced behavioral and zif268 mRNA gene expression in the dorsal and ventral striatum. Peltier & coworkers (1994) demonstrated that a low dose of RIT (1.0 mg/kg) had no significant effect on the dose-response curve for cocaine self-administration. Previous studies demonstrated that zif268 mRNA is expressed at a higher basal level in rat brains (Christy et al, 1988; Worley et al, 1991). Psychostimulants, such as cocaine and amphetamine, which are dopamine reuptake blockers, immediately induced dramatic increase in zif268 mRNA expression in the CPu and NAc of rat brain (Moratalla et al, 1992; Ennulat et al, 1994). Bhat & Baraban (1993) demonstrated that transcription factor gene expression induced by cocaine appears to be mediated by pharmacologically defined D1 dopamine receptors. They also showed that the induction of immediate early gene expression in the striatum is due to the contribution of both dopaminergic and serotonergic systems. These data suggest that both systems are required for behavioral and genomic responsiveness by cocaine. The fact that zif268 mRNA expression and behavior alterations induced by cocaine was not decreased by RIT suggests that the antagonism of 5HT2/1C receptors alone is not sufficient to decrease cocaine-induced zif268 mRNA expression and behavioral changes. This conclusion would be supported by the finding that amperozide, another 5HT2 receptor antagonist which also acts on dopaminergic neurons, significantly blocked cocaine-induced behavioral alterations and c-fos and zif268 mRNA expressions in the striatum (Choe & Kim, 2000). Thus, interaction of the dopaminergic- and serotonergic system in the striatum would mediate behavioral changes and zif268 mRNA expression induced by cocaine via unknown mechanism.

In conclusion, 5HT2/1C receptors in the dorsal and ventral striatum may not be involved in zif268 mRNA expression as well as behavioral alterations induced by a single injection of cocaine. Further study would be required to determine whether dopamine receptors are involved in cocaine-induced behavior and immediate early gene expression in relation to 5HT2/1C receptors in the striatum.

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