ELSEVIER

Pharmacology, Biochemistry and Behavior 69 (2001) 225–232

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Attenuation of cocaine-induced genomic and functional responses in prenatal cocaine-exposed rabbits

N. Tilakaratne, G. Cai, E. Friedman*

Laboratory of Molecular Pharmacology, Department of Pharmacology and Physiology, MCP Hahnemann School of Medicine, 245 N 15th Street, Philadelphia, PA 19102, USA

Received 14 April 2000; received in revised form 23 January 2001; accepted 19 February 2001

Abstract

The effects of in utero cocaine exposure on cocaine-induced genomic and functional responses in postnatal life were examined. Pregnant Dutch Belted rabbits were injected intravenously, twice daily, with cocaine hydrochloride (4 mg/kg) or saline from day 8 through day 29 of pregnancy. Prenatally exposed kits were challenged with cocaine on postnatal day 20. In prenatal saline-exposed kits, cocaine induced time- and dose-dependent c-*fos* gene expression in both frontal cortex and striatum. Prenatal cocaine exposure reduced cocaine-induced c-*fos* responses by 35–58% in the frontal cortex and 37–41% in the striatum. Cocaine-induced functional responses that included head bobbing, seizure, and locomotor activity were also attenuated in prenatal cocaine-exposed kits. Cocaine-induced c-*fos* expression and functional responses were blocked by the D_1 dopamine receptor antagonist, SCH23390, or by the serotonin receptor antagonist, methysergide, but not by the D_2 dopamine receptor antagonist, L-sulpride. The results indicate that in utero cocaine exposure leads to diminished responses to cocaine challenge in the offspring, which may be mediated by prenatal cocaine-induced alterations in one or more components of the D_1 dopamine and/or serotonin receptor signaling systems during early postnatal life.

Keywords: Brain; Prenatal cocaine; c-*fos*; Functional response; Rabbit

1. Introduction

Prenatal exposure to cocaine has been shown to result in developmental and neurobehavioral abnormalities in the newborn (Chasnoff et al., 1992; Zuckerman et al., 1989). Among the frequently observed neurologic abnormalities in cocaine-exposed infants are tremors, hypertonia, and impaired motor and sensory development (Chiriboga et al., 1993). Studies using animal models of cocaine use during pregnancy support the clinical and epidemiological data on behavioral and cognitive deficits in the offspring (Kosofsky et al., 1994; Levitt et al., 1997; Molina et al., 1994; Sobrian et al., 1990). Additionally, they have provided insight into the molecular mechanism of the adverse effects of cocaine on the developing central nervous system (Cabrera et al., 1993; Keller et al., 1994; Levitt et al., 1997).

Systemic administration of cocaine elicits a rapid induction of expression of immediate-early genes such as c-*fos*, c-*jun*, *zif* 268 in the stratum, limbic forebrain, and nucleus accumbens of adult rodents (Graybiel et al., 1990; Helton et al., 1993; Johansson et al., 1994; Steiner and Gerfen, 1993; Wang et al., 1996). Both dopamine and serotonin receptor systems have been recognized as mediators of these effects (Bhat and Baraban, 1993; Humblot et al., 1998; Meyer et al., 1992; Young et al., 1991). Immediate-early gene products function as transcription factors that regulate the expression of one or more target genes. The orchestration of immediate-early gene expression and subsequent target gene activity has been suggested as the possible mechanism mediating the short and long-term effects of central stimulants (Graybiel et al., 1990). Consequently, the rapid stimulation of immediate-early gene expression is regarded as a useful indicator of neuronal activation in a variety of central nervous system stimulation paradigms (Sheng and Greenberg, 1990).

In rodents, acute cocaine elicits a variety of behavioral responses; predominant among them are increased sniffing, rearing, and locomotion (Spear et al., 1989). Cocaine acts by binding to and inhibiting presynaptic dopamine, serotonin,

* Corresponding author. Tel.: +1-215-762-2396; fax: +1-215-762-2299.

E-mail address: eitan.friedman@drexel.edu (E. Friedman).

0091-3057/01/$ – see front matter
PII: S0091-3057(01)00534-2

and norepinephrine transporters with consequent increases in the synaptic concentrations of these neurotransmitters. Most studies attribute a dominant role to the mesocorticolimbic dopaminergic system in mediating cocaine's action. Selective D_1 dopamine receptor antagonists, destruction of dopamine innervation of the nucleus accumbens, or mutations of the D_1 dopamine receptor abolish many of cocaine's functional and behavioral effects (Cabib et al., 1991; Xu et al., 1994). Nevertheless, there is also evidence implicating the 5-HT system in the stimulant actions of cocaine (Bhat and Baraban, 1993).

Neurological abnormalities resulting from prenatal exposure to cocaine may stem from alterations to one or more of these neurotransmitter systems. Since activating these pathways elicits immediate-early gene responses, changes in cocaine-induced genomic responses may indicate developmental alterations attributable to prenatal cocaine exposure. In this study, rabbit kits born to dams that were treated chronically with cocaine during pregnancy were used to determine the effects of prenatal cocaine on the sensitivity to the drug in early postnatal life. The results show that prenatal exposure to cocaine blunts both genomic and functional responses to postnatal cocaine challenge.

2. Methods

2.1. Animals and prenatal cocaine exposure

Dutch Belted rabbits, of confirmed breeding history, were obtained from Myrtle Rabbitry (Thompson Station, TN) and maintained, singly caged with ad libitum supply of food (laboratory rabbit chow HF/5325) and water. Room temperature was set at $21 \pm 1^{\circ}C$ with a 12-h light–dark cycle (light on at 7:00 a.m. and off at 7:00 p.m.). After a week of acclimation to the housing facility, the females were bred with bucks of the same breed. Pregnant dams were randomly distributed between two treatment groups: cocaine-treated and saline-treated controls. Rabbits in the cocaine-treated group were injected intravenously, twice daily, with 4 mg/kg cocaine hydrochloride (NIDA, Bethesda, MD) via the marginal ear vein from Day 8 through Day 29 of gestation. The injections were administered between 8:00 and 8:30 a.m. and between 3:00 and 3:30 p.m. each day. Control dams were injected with an equal volume of saline (2 ml). This regimen of cocaine administration had no significant effects on the weight gain of the dams or the duration of pregnancy as well as no effects on the kits as measured by litter size, survival rate, frequency of birth defects, or body weight (Murphy et al., 1995, 1997; Wang et al., 1995). The kits were nursed by their own mother until Day 20 when they were removed for experimentation. We have chosen Day 20 animals as the test subjects, as prior investigations in this model have shown neurochemical and morphological changes that persist from gestational to postnatal life (Friedman et al., 1996; Jones et al., 2000).

2.2. Postnatal acute cocaine challenge

c-*fos* gene expression was assessed in 20-day-old kits 15 to 150 min following intraperitoneal saline or cocaine challenge. The animals were decapitated and frontal (medial prefrontal and anterior cingulated) cortical and striatal tissues were dissected from each brain and stored individually on dry ice until they were processed for RNA extraction, approximately 2 h later. No more than one kit per litter was included in each treatment group. Sex of each sacrificed animal was determined by examining the internal sex organs.

2.3. RNA extraction and analysis

Total RNA from individual brain regions was obtained by the guanidine isothiocyanate–phenol extraction method (Chomczynski and Sacchi, 1987). Ten micrograms of RNA from each tissue was size-fractionated by electrophoresis on 1% agarose–6% formaldehyde gel, stained with ethidium bromide, and visualized to confirm equal loading across lanes. RNA on the gel was transferred onto a Nytran (Schleicher & Schuell, Keene, NH) membrane by capillary transfer and fixed by ultraviolet irradiation (Stratalinker, Stratagene, CA). Following 2-h prehybridization at 42°C in a solution containing 50% formamide, 0.75 M NaCl, 0.075 M sodium citrate, Denhardt's solution (0.02% bovine serum albumin, 0.2% polyvinylpyrrolidone, 0.2% ficoll), 0.1% sodium dodecyl sulfate (SDS), hybridization was carried out for 20 h, at 42°C, in the same solution with additions of dextran sulfate (0.1 g/ml), denatured salmon sperm DNA (100 g/ml), and ^{32}P-labeled DNA probe (10×10^6 cpm). The probe used for the detection of c-*fos* mRNA was a 500 bp NcoI–AccI restriction fragment of the c-*fos* cDNA. Posthybridization washes were as follows: once at room temperature for 15 min in solution containing 0.3 M NaCl, 0.03 M sodium citrate, and 0.1% SDS and once at 55°C for 15 min in solution containing 0.03 M NaCl, 0.003 M sodium citrate, and 0.1% SDS. Membrane was exposed to X-ray film with intensifying screen at $-70^{\circ}C$ for 3 to 5 days. The relevant autoradiographic signals were quantitated by laser scan densitometry (Biomed Instruments, Fullerton, CA). Slot-blot analysis was done using a slot-blot apparatus (Schleicher & Scheull) according to the manufacturer's protocol. Briefly, 10 μg total RNA per slot was applied onto Nytran membrane, and the bound RNA fixed by ultraviolet irradiation. Hybridization to a ^{32}P-labeled c-*fos* cDNA probe, autoradiography, and quantitation of the hybridization signals were similar to those employed in the Northern analyses.

2.4. Functional response test

Responses to cocaine challenge were monitored for 20 min by placing the rabbit kits, immediately after the injection, on a bench top lined with corrugated paper. Responses were scored as follows: (0) no response: no

apparent change in behavior; (1) minimal response: running in circles along the perimeter of the bench-top and occasional head bobbing; (2) mild response: frequent head-bobbing, initial running activity that quickly changed into still posture with stiffened and extended limbs, but without convulsions, followed by complete recovery in 5–8 min; or (3) severe response: initial hyperactivity quickly followed by ataxia, falling on the side or rolling over, violent convulsions and peddling motion of all four limbs, excessive salivation, and delayed (>10 min) recovery.

2.5. Pretreatment with dopaminergic or serotonergic antagonists

A separate experiment was conducted to determine the effect of pretreatment with the D_1 dopamine receptor antagonist, SCH23390 (Schering, Bloomfield, NJ), the D_2 dopamine receptor antagonist, sulpiride (Ravizza, Milan, Italy), or the 5-hydroxytryptamine (5-HT) serotonin receptor antagonist, methysergide (Sandoz, East Hanover, NJ) on the response to an acute cocaine challenge. Prenatal saline- and cocaine-exposed kits were injected intraperitoneally with one of the following combinations of two drugs administrated 30 min apart: (a) two injections of saline, (b) saline followed by cocaine (40 mg/kg), (c) SCH23390 (0.075 mg/kg) followed by saline, (d) L-sulpiride (50 mg/kg) followed by saline, (e) methysergide (5 mg/kg) followed by saline, (f) SCH23390 followed by cocaine, (g) L-sulpiride followed by cocaine, or (h) methysergide followed by cocaine. The animals were monitored and scored as described above. Animals were sacrificed 60 min after the second injection and tissues were collected for RNA extraction and analysis.

2.6. Statistical analysis

Data obtained from four to eight individual experiments per group were presented as mean ± S.E.M. The data for c-*fos* expression and functional responses were analyzed by the two-factor analysis of variance (ANOVA) followed by the Newman–Keuls test. The two-factors used to analyze the time-course, dose–response and receptor-antagonist data were the challenge treatment and time point, prenatal exposure and acute cocaine dose, and prenatal exposure and antagonist treatment, respectively. Statistical significance was set at the level of $P<.05$.

3. Results

3.1. Cocaine-induced time-dependent c-fos gene expression

Northern analysis demonstrated that acute intraperitoneal injection of cocaine hydrochloride (30 mg/kg) time-dependently induced significant c-*fos* mRNA gene expressions in both the frontal cortex and striatum of 20-day-old control kits. As shown in Fig. 1, the response became significant at 30 min, peaked at 60 min, and returned to basal levels 150 min after the administration of cocaine. In both cortex and striatum, the induction of c-*fos* mRNA peaked at about 400% above the respective levels in the saline controls.

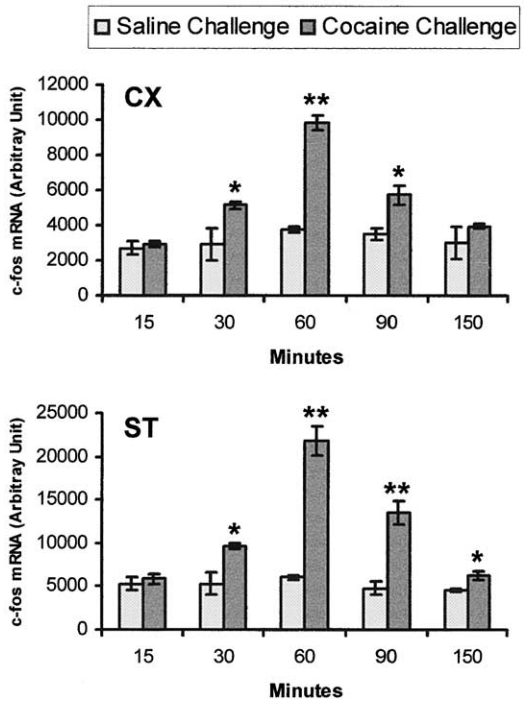

Fig. 1. Time-course analysis of cocaine-induced c-*fos* mRNA expression in 20-day-old prenatal saline-treated rabbit kits. Animals were administered a single intraperitoneal injection of saline or 30 mg/kg cocaine and sacrificed at the indicated postinjection time-points. Frontal cortex (CX) and striatum (ST) were dissected, and processed for RNA extraction. Ten micrograms of total RNA from each animal was size-fractionated by agarose gel electrophoresis, transferred onto a nylon-based membrane, and analyzed by Northern hybridization to a ^{32}P-labeled c-*fos* cDNA probe. The corresponding autoradiograph signals were quantitated by laser scan densitometry. $*P<.05$, $**P<.01$ compared with the respective saline challenge.

3.2. Effect of prenatal cocaine exposure on cocaine-induced c-fos gene expression

The effects of prenatal cocaine exposure on acute cocaine-induced c-*fos* mRNA expression were examined in 20-day-old kits. As shown in Fig. 2, cocaine challenge dose-dependently increased c-*fos* mRNA in the frontal cortex and striatum of prenatal saline or cocaine-exposed rabbits. However, the responses to the cocaine challenge in prenatal cocaine-exposed kits were lower by 35% to 58% in the cortex and 37% to 41% in the striatum when compared with responses to the three cocaine doses (20, 30, or 40 mg/kg) tested in the control prenatal saline-treated offsprings ($P<.05$, ANOVA, in both brain regions).

In order to determine the effect of gender or litter on cocaine-induced c-*fos* gene expression, the data on the effect of acute cocaine (30 mg/kg) on c-*fos* gene expression in prenatal saline- or cocaine-exposed kits were segregated by

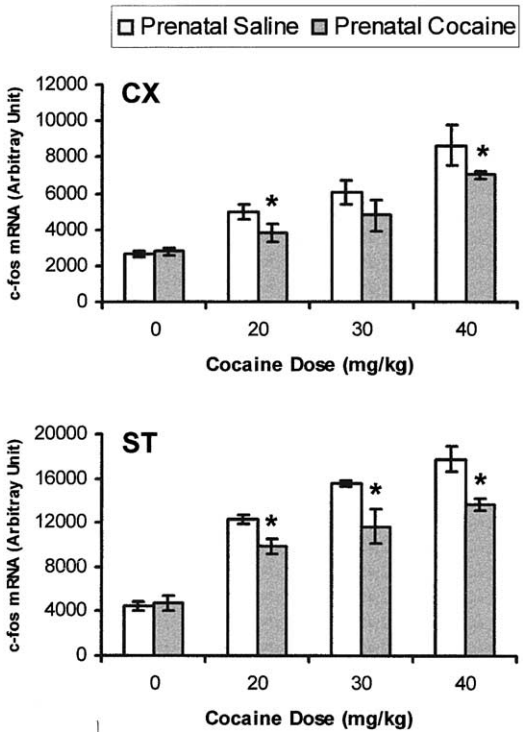

Fig. 2. Dose–response of c-*fos* mRNA to acute cocaine in 20-day-old prenatal saline- or cocaine-exposed rabbit kits. Animals received intraperitoneally 20, 30, or 40 mg/kg cocaine. Animals were sacrificed 60 min postinjection, and frontal cortex (CX) and striatum (ST) were dissected from each brain. RNA was extracted from each brain sample and 10 μg of total RNA was size-fractionated on agarose gel, transferred on to a nylon based membrane, and hybridized to a ^{32}P-labeled c-*fos* cDNA probe. The corresponding autoradiograph signals were quantitated by laser scan densitometry. * $P<.05$ compared with prenatal saline-exposed kits within each dose of cocaine.

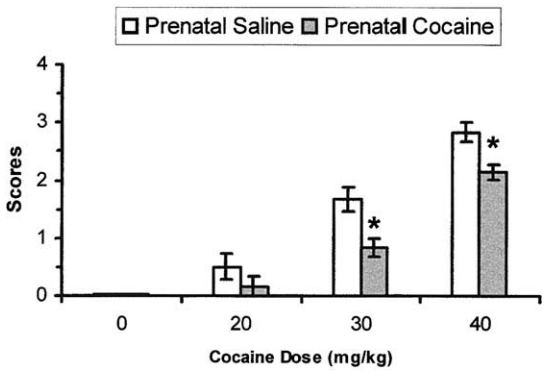

Fig. 3. Dose-dependent functional responses to cocaine challenge in 20-day-old prenatal saline or cocaine-exposed rabbit kits (20 days old). Animals were given a single intraperitoneal injection of 20, 30, or 40 mg/kg cocaine hydrochloride and their responses were monitored for 20 min following injection. Animals were assigned to one of four reaction scores (0, 1, 2, or 3) based on the severity of the response as described in the Methods section. Each bar represents mean ± S.E.M. of the reaction scores for each group. * $P<.05$ compared with prenatal saline-exposed kits within each dose of cocaine.

sex or litter of origin. Group comparisons to determine the significance of prenatal treatment, sex, or litter of origin revealed that while the prenatal treatment significantly influenced the c-*fos* response ($P<.05$ for either cortex or striatum), sex or litter of origin had no significant effect on the responses to the cocaine challenge (data not shown).

3.3. Effect of prenatal cocaine exposure on cocaine-induced functional responses

Cocaine-induced functional responses in 20-day-old rabbits are summarized in Fig. 3. Acute cocaine challenge dose-dependently induced functional responses in both prenatal saline and prenatal cocaine animals. At the dose of 20 mg/kg, cocaine elicited significant but minimal responses in prenatal saline rabbits. The functional responses to 40 mg/kg cocaine

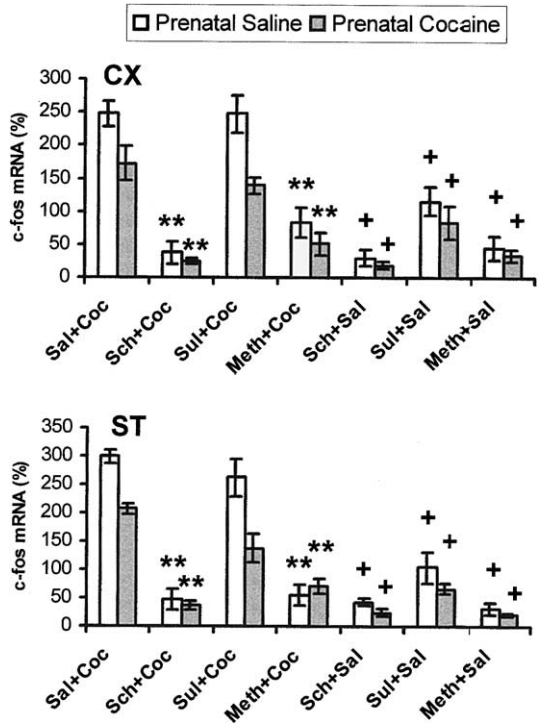

Fig. 4. Effect of dopaminergic and serotonergic antagonists on cocaine-induced c-*fos* mRNA in 20-day-old prenatal saline or prenatal cocaine-exposed rabbit kits. Animals representing the two prenatal treatment groups were given one of the following combinations of intraperitoneal injections administered 30 min apart: (a) saline (SAL)–saline, (b) saline–40 mg/kg cocaine (COC), (c) 0.075 mg/kg SCH23390 (Sch)–40 mg/kg cocaine, (d) 50 mg/kg L-sulpiride (Sul)–40 mg/kg cocaine, (e) 5 mg/kg methysergide (Meth)–40 mg/kg cocaine, (f) 0.075 mg/kg SCH23390–saline, (g) 50 mg/kg L-sulpiride–saline, or (h) 5 mg/kg methysergide–saline. Animals were sacrificed 60 min after the second injection, the frontal cortex (CX) and striatum (ST) were dissected from each brain and RNA extracted. Ten micrograms of total RNA from each tissue was size-fractionated by agarose gel electrophoresis, transferred onto nylon-based membrane, and hybridized to a ^{32}P-labeled c-*fos* cDNA probe. The corresponding autoradiograph signals were quantitated by laser scan densitometry. Data represent a percent increase in c-*fos* mRNA over that in the saline–saline group. ** $P<.01$ compared with respective saline–cocaine treated kits. $^{+}P<.05$ compared with respective saline–saline treated kits.

challenge were severe. Acute cocaine-induced functional responses were greatly attenuated across the cocaine doses in prenatal cocaine rabbits compared to those in prenatal saline animals. The prenatal cocaine animals did not response to the challenge of 20 mg/kg cocaine. At the acute cocaine challenge doses of 30 or 40 mg/kg, the responses were significantly reduced in rabbit offsprings that were prenatally exposed to cocaine ($P < .05$, ANOVA).

3.4. Effects of dopamine- or serotonin-receptor antagonists on cocaine-induced responses

In order to characterize the c-*fos* responses to cocaine in rabbit kits, the effects of the D_1 and D_2 dopamine receptor antagonists, SCH23390 (0.075 mg/kg) and L-sulpiride (50 mg/kg), or the 5-HT serotonin receptor antagonist, methysergide (5 mg/kg), were tested. As shown in Fig. 4, SCH23390 or methysergide significantly reduced cocaine-induced c-*fos* gene expression in the cortex and striatum of prenatal saline animals (86% and 87% by SCH23390 and 68% and 74% by methysergide). L-Sulpiride did not affect cocaine-induced c-*fos* mRNA expression. However, L-sulpiride alone elicited a 2-fold stimulation of the c-*fos* response in both brain regions. SCH23390 or methysergide, when injected alone, elicited small 20% to 30% increases in c-*fos* expression.

The functional responses to cocaine were also examined in prenatal saline animals after pretreatment with the dopamine or serotonin receptor antagonists (Fig. 5). The responses to cocaine challenge were completely suppressed in all animals that were pretreated with methysergide. The D_1 dopamine receptor antagonist, SCH23390, also markedly suppressed the severity of the behavioral responses to cocaine, while the D_2 receptor selective antagonist, L-sulpiride, was without effect. SCH23390, L-sulpiride, or methysergide, when injected alone, did not elicit significant responses.

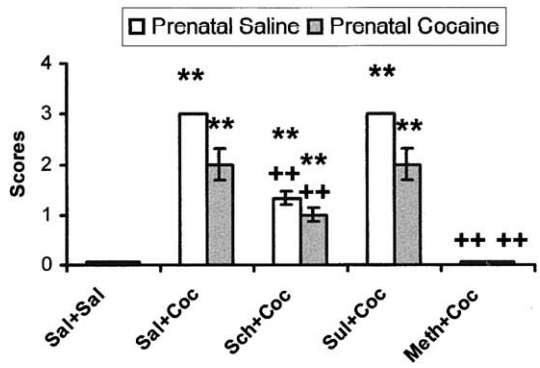

Fig. 5. Effect of dopaminergic and serotonergic antagonists on cocaine-induced functional responses in 20-day-old prenatal saline or prenatal cocaine exposed rabbit kits. Animals were given one of the following combinations of treatments 30 min apart: (a) two injections of saline, (b) saline–40 mg/kg cocaine (Sal+Coc), (c) 0.075 mg/kg SCH23390–40 mg/kg cocaine (SCH+Coc), (d) 50 mg/kg L-sulpiride–40 mg/kg cocaine (Sul+Coc), (e) 5 mg/kg methysergide–40 mg/kg cocaine (Meth+Coc), (f) 0.075 mg/kg SCH23390–saline (SCH+Sal), (g) 50 mg/kg L-sulpiride–saline (Sul+Sal), and (h) 5 mg/kg methysergide–saline (Meth+Sal) and their functional responses were monitored for 20 min after the second injection. The severity of the responses were scored on a scale of 0 to 3 as described in the Methods section. Each bar represents mean ± S.E.M. of the reaction scores for each group. $^{**}P < .01$ compared with saline–saline group; $^{++}P < .01$ compared with saline–cocaine group.

Cocaine-induced c-*fos* gene expression and functional responses tested in 20-day-old rabbits that were exposed prenatally to cocaine were also markedly antagonized by SCH23390 or methysergide but not by L-sulpiride (Figs. 4 and 5).

4. Discussion

The present results demonstrate that acute cocaine injection stimulates c-*fos* gene expression in the neonatal rabbit brain frontal cortex and striatum. The response is dose- and time-dependent, evident as early as 30 min posttreatment, peaks at 60 min, and returns to near basal levels at 150 min. Gender or litter of origin did not influence the c-*fos* gene responses to cocaine in prenatal saline- or cocaine-exposed rabbits. The finding of cocaine-induced c-*fos* gene expression in rabbit brain during early postnatal age is an extension of similar findings previously demonstrated in adult rodents (Graybiel et al., 1990; Helton et al., 1993; Johansson et al., 1994; Steiner and Gerfen, 1993; Weaver et al., 1995). The relatively high level of basal c-*fos* expression in rabbit neonates is another noteworthy feature of these results. Immediate-early genes, in adult subjects, are not constitutively expressed in most organs, but are rapidly and robustly activated in response to a variety of stimuli. The relatively high constitutive c-*fos* expression in early postnatal brains seen in the present study may be related to their active developmental status. Alcantara and Greenough (1993) and Kosofsky et al. (1995) have shown considerable basal c-*fos* expression in rat brain during the first 2 weeks of life that are subject to significant regional and cellular variation. Alternatively, since c-*fos* expression is also stimulated by acute stress, the relatively high expression level in control animals may reflect a greater sensitivity to handling stress in the newborn.

The apparent ability of SCH23390 to suppress the enhanced expression of c-*fos* induced by cocaine suggests that the effect is mediated, at least in part, by D_1 dopamine receptors. This is in agreement with previous results obtained in mice and rats (Cole et al., 1992; Young et al., 1991). However, the present results also demonstrate that methysergide, a serotonin receptor antagonist, also blocks the c-*fos* response to a similar extent as SCH23390, suggesting that the serotonergic system contributes to the response to cocaine challenge. These findings are consistent with the results of Bhat and Baraban (1993) who reported on a synergism between the serotonergic and dopaminergic systems in mediating the actions of cocaine. Furthermore, a recent study demonstrates marked reduction in cocaine-

induced c-*fos* gene expression in 5-HT_{1B} receptor knockout mice, suggesting that the 5-HT_{1B} serotonin receptors mediate this cellular response to cocaine (Lucas et al., 1997). Although immediate-early gene induction with indirect dopaminergic agonists such as cocaine has been demonstrated, the effects of selective direct D_1 or D_2 agonists on immediate-early gene stimulation is found to be inconsistent. While some studies have demonstrated a dramatic c-*fos* response to direct activation of D_1 dopamine receptors with SKF38393 (Robertson et al., 1989), others have indicated the requirement of prolonged stimulation of both D_1 and D_2 dopamine receptors suggesting a synergistic activity between these two dopamine receptor subtypes (LaHoste et al., 1993). However, the present data demonstrate that cocaine-induced c-*fos* gene expression is completely prevented by a selective D_1 dopamine receptor antagonist but not by a selective D_2 dopamine receptor antagonist, suggesting a dominant role of D_1 dopamine receptors in mediating this response to cocaine. Thus, the genomic response to cocaine in the 20-day-old rabbit is dependent on activation of D_1 dopamine and serotonin receptors. Given the ability of cocaine to block nerve terminal neurotransmitter transporters of both dopamine and serotonin neurons, it appears that both of these synapses are involved in this action of cocaine.

Acute exposure of adult rodents to cocaine results in several characteristic responses including locomotion, stereotypic behavior, and seizure activity (Woolverton and Johnson, 1992). The response of 20-day-old rabbit kits observed in the present study are similar to those seen in adult rodents, with the notable exception that the stimulant action in the rodent lasts for 20 min or more (Sobrian et al., 1990), while in the young rabbit the response is comparatively of very short duration (2 to 3 min), even at the relatively high doses of cocaine employed in the present study. It is not clear whether species, age, or other determinants such as differences in plasma esterase activity are responsible for this difference. The functional response to cocaine in the present work was completely abolished by methysergide, drastically reduced by SCH23390, and unaffected by L-sulpiride pretreatment — findings that further support the role of serotonin and of D_1 dopamine receptors in the actions of cocaine in the neonatal rabbits.

The present results also demonstrate that intrauterine exposure to cocaine alters the sensitivity of the offspring, during early postnatal life, to the genomic and functional responses to cocaine challenge. The genomic responses to cocaine were blunted both in the frontal cortex and in striatum, across all tested cocaine doses. Stimulant-induced expression of c-*fos* and other immediate-early genes results from the cumulative action of signals initiated by receptor stimulation. This action is widely regarded to be part of the mechanism that mediates short- and long-term effects of neurotransmitter receptor activation (Sheng and Greenberg, 1990). The more striking observation with regard to response, however, is the marked reduction in sensitivity to the locomotor, head-bobbing, and seizure-inducing effects of cocaine in rabbit kits that had been prenatally exposed to the drug. Thus, the c-*fos* and functional responses to acute cocaine in prenatal cocaine-exposed kits were consistently lower in severity to that elicited in prenatal saline kits at age 20 days. In light of our previous studies demonstrating that the effects of prenatal cocaine exposure on D_1 dopamine receptor signaling and on cortical neuronal structure appear as early as gestational Day 20 and persist into adulthood (Friedman et al., 1996; Jones et al., 2000), the attenuated cocaine-mediated responses to cocaine noted in the present experiment may therefore be representative of a sustained change in brain neurochemistry that is elicited during gestation. The desensitization to cocaine noted in the present study parallels that reported by Simansky and Kachelries (1996). These authors found a suppression of amphetamine-induced head-bobbing in both the young and adult progeny of cocaine-treated does. The present results in lagomorphs are also consistent with several previous reports which demonstrated decreased responsiveness to cocaine following prenatal exposure to the drug in mice and rats (Byrnes et al., 1993; Meyer et al., 1992, 1994). However, enhanced behavioral response to cocaine in prenatal cocaine-exposed adult rats was also reported previously (Peris et al., 1992).

The neurochemical basis of desensitization to stimulants is presently not well understood. In view of the roles of the dopaminergic and serotonergic systems in mediating the actions of cocaine, potential changes in these neurotransmission systems are of particular interest. Dopaminergic changes resulting from prenatal cocaine exposure include reduced basal dopamine levels in the striatum (Weise-Meyer et al., 1993), increased striatal dopamine transporter (Koff and Miller, 1994), decreased spontaneous dopaminergic cell activity (Minabe et al., 1992), and reduced D_1 dopamine receptor/Gαs coupling (Friedman et al., 1996; Wang et al., 1995). Similarly, a number of serotonergic deficiencies have been reported to result from prenatal cocaine exposure (Akbari et al., 1992). However, prior investigations performed in the present animal models do not support the involvement of a presynaptic mechanism since dopamine levels, tyrosine hydroxylase activity, and the number of dopamine uptake sites are unaltered in these in utero cocaine-exposed rabbits (Du et al., 1999; Wang et al., 1996). In addition, neither dopamine nor serotonin receptor number seem to be affected by prenatal cocaine exposure (Cabrera et al., 1993; De Bartolomeis et al., 1994; Wang et al., 1995). Since intracellular second messengers are regulated by dopamine and serotonin receptor activity, the changes in neurochemistry and/or in postreceptor mechanisms resulting from prenatal cocaine exposure may be the basis for the reduced responsiveness to acute cocaine challenge reported in this communication.

In conclusion, results from the present studies demonstrate that rabbit offsprings exposed in utero to cocaine are less responsive to functional and genomic effects of acute

cocaine when challenged during early postnatal life. The results also suggest that both dopamine and serotonin neuronal systems contribute to the effects of cocaine. The impact of prenatal cocaine on the development of these two neurotransmitter systems may account for the lasting decreased responsiveness to cocaine in the offspring.

Acknowledgments

This research was supported by USPHS Grant No. DA 11029 from the National Institute on Drug Abuse and a grant from the March of Dimes Birth Defects Foundation.

References

Alcantara AA, Greenough WT. Developmental regulation of Fos and Fos-related antigens in cerebral cortex, striatum, hippocampus, and cerebellum of the rat. J Comp Neurol 1993;334:75–85.

Akbari HM, Kramer HK, Whitaker-Azmitia PM, Spear LP, Azmitia EC. Prenatal cocaine exposure disrupts the development of the serotonergic system. Brain Res 1992;572:57–63.

Bhat RV, Baraban JM. Activation of transcription factor genes in the striatum by cocaine: role of both serotonin and dopamine systems. J Pharmacol Exp Ther 1993;267:496–505.

Byrnes JJ, Pritchard GA, Koff JM, Miller LG. Prenatal cocaine exposure: decreased sensitization to cocaine and decreased striatal dopamine transporter binding in offspring. Neuropharmacology 1993;32:721–3.

Cabib S, Castellano C, Cestari V, Fillibeck U, Puglisis-Allegra S. D_1 and D_2 receptor antagonists differentially affect cocaine-induced locomotor hyperactivity in the mouse. Psychopharmacology 1991;105:335–9.

Cabrera TM, Yracheta JM, Li Q, Levy AD, VandeKar LD, Battaglia G. Prenatal cocaine produces deficits in serotonin mediated neuroendocrine responses in adult rat progeny: evidence for long-term functional alterations in brain serotonin pathways. Synapse 1993;15:158–68.

Chasnoff IJ, Freier C, Murray J. Cocaine/polydrug use in pregnancy: two-year follow-up. Pediatrics 1992;89:284–9.

Chiriboga CA, Bateman DA, Brust JC, Hauser WA. Neurologic findings in neonates with intrauterine cocaine exposure. Pediatr Neurol 1993;9:115–9.

Chomczynski P, Sacchi N. Single-step method of RNA isolation by guanidinium thiocyanate–phenol–chloroform extraction. Anal Biochem 1987;162:156–9.

Cole AJ, Bhat RV, Patt C, Worley PF, Baraban JM. D_1 dopamine receptor activation of multiple transcription factor genes in rat striatum. J Neurochem 1992;58:1420–6.

de Bartolomeis A, Austin MC, Goodwin GA, Spear LP, Pickar D, Crawley JN. Dopaminergic and peptidergic mRNA levels in juvenile rat brains after prenatal cocaine treatment. Mol Brain Res 1994;21:321–32.

Du W, Aloyo VJ, Pazdelski PS, Harvey JA. Effects of prenatal cocaine exposure on amphetamine-induced dopamine release in the caudate nucleus of the adult rabbit. Brain Res 1999;836:194–8.

Friedman E, Yadin E, Wang HY. Effect of prenatal cocaine on dopamine receptor-G coupling in mesocortical regions of the rabbit brain. Neuroscience 1996;70:739–47.

Graybiel AM, Moratalla R, Robertson H. Amphetamine and cocaine induce drug-specific activation of the c-*fos* gene in strosome-matrix compartments and limbic subdivisions of the striatum. Proc Natl Acad Sci USA 1990;87:6912–6.

Helton TE, Daunais JB, McGinty JF. Convulsant doses of cocaine alter immediate-early gene and opioid peptide expression in rat limbic forebrain. Mol Brain Res 1993;20:285–8.

Humblot N, Thiriet N, Gobaille S, Aunis D, Zwiller J. The serotonergic system modulates the cocaine-induced expression of the immediate early genes *egr*-1 and c-*fos* in rat brain. Ann NY Acad Sci 1998;844:7–20.

Johansson B, Lindstrom K, Fredholm BB. Differences in the regional and cellular localization of c-*fos* messenger RNA induced by amphetamine, cocaine and caffeine in the rat. Neuroscience 1994;59:837–49.

Jones LB, Stanwood GD, Reinoso BS, Washington RA, Wang HY, Friedamn E, Levitt P. In utero cocaine-induced dysfunction of D1 receptor signaling and abnormal differentiation of cerebral cortical neurons. J Neurosci 2000;20:4606–14.

Keller RW, Maisonneuve IM, Nuccio DM, Carlson JN, Glick SD. Effects of prenatal cocaine exposure on the nigrostriatal dopamine system: an in-vivo microdialysis study in the rat. Brain Res 1994;634:266–74.

Koff JM, Miller LG. Prenatal cocaine exposure increased striatal dopamine transporter binding in offspring at 3 and 6 months of age. Brain Res Bull 1994;33:223–4.

Kosofsky BE, Wilkins AS, Gressens P, Evrard P. Transplacental cocaine exposure: a mouse model demonstrating neuroanatomic and behavioral abnormalities. J Child Neurol 1994;9:234–41.

Kosofsky BE, Genova LM, Hyman SE. Prenatal age defines specificity of immediate early gene induction by cocaine in developing rat brain. J Comp Neurol 1995;351:27–40.

LaHoste GJ, Yu J, Marshall JF. Striatal *fos* expression is indicative of dopamine D_1/D_2 synergism and receptor supersensitivity. Proc Natl Acad Sci USA 1993;90:7451–5.

Levitt P, Harvey JA, Friedman E, Simansky KJ, Murphy EH. Prenatal cocaine exposure: new evidence for neurotransmitter influences on brain development. Trends Neurosci 1997;20:269–74.

Lucas JJ, Segu L, Hen R. 5-Hydroxytryptamine 1B receptors modulate the effect of cocaine and c-*fos* expression: converging evidence using 5-hydroxytryptamine 1B knockout mice and the 5-hydroxytryptamine 1B/1D antagonist GR127936. Mol Pharmacol 1997;51:755–63.

Meyer JS, Sherlock JD, Macdonald NR. Effects of prenatal cocaine exposure on behavioral response to a cocaine challenge on postnatal day 11. Neurotoxicol Teratol 1992;14:183–9.

Meyer JS, Robinson P, Todtenkopf MS. Prenatal cocaine treatment reduces haloperidol-induced catalepsy on postnatal day 10. Neurotoxicol Teratol 1994;16:193–9.

Minabe Y, Ashby CR, Heyser C, Spear LP, Wang RY. The effects of prenatal cocaine exposure on spontaneously active midbrain dopamine neurons in adult male offspring: an electrophysiological study. Brain Res 1992;586:152–6.

Molina VA, Wagner JM, Spear LP. The behavioral response to stress is altered in adult rats exposed prenatally to cocaine. Physiol Behav 1994;55:941–5.

Murphy EH, Hammer JG, Schumann MD, Groce MY, Wang XH, Jones L, Romano AG, Harvey JA. The rabbit as a model for studies of cocaine exposure in utero. Lab Anim Sci 1995;45:163–8.

Murphy EH, Fischer I, Friedman E, Grayson D, Jones L, Levitt P, O'Brien-Jenkins A, Wang HY, Wang XH. Cocaine administration in pregnant rabbits alters cortical structure and function in their progeny in the absence of maternal seizures. Exp Brain Res 1997;114:433–41.

Peris J, Coleman-Hardee M, Millard WJ. Cocaine in utero enhances the behavioral response to cocaine in adult rats. Pharmacol, Biochem Behav 1992;42:509–15.

Robertson HA, Peterson MR, Murphy K, Robertson GS. D_1-dopamine receptor agonists selectively activate striatal c-*fos* independent of rotational behavior. Brain Res 1989;503:346–9.

Sheng M, Greenberg ME. The regulation and function of c-*fos* and other immediate-early genes in the nervous system. Neuron 1990;4:478–85.

Simansky KJ, Kachelries WJ. Prenatal exposure to cocaine selectively disrupts motor responding to D-amphetamine in young and mature rabbits. Neuropharmacology 1996;35:71–8.

Sobrian SK, Burton LE, Robinson NL, Ashe WK, James H, Stokes DL, Turner LM. Neurobehavioral and immunological effects of prenatal cocaine exposure in rat. Pharmacol, Biochem Behav 1990;35:617–29.

Spear LP, Kirstein CL, Frambes NA. Cocaine effects on the developing

central nervous system: behavioral, psychopharmacological and neurochemical studies. Ann NY Acad Sci 1989;562:290–307.

Steiner H, Gerfen CR. Cocaine-induced c-*fos* messenger RNA is inversely related to dynorphin expression in striatum. J Neurosci 1993;13:5066–81.

Wang HY, Runyan S, Yadin E, Friedman E. Prenatal exposure to cocaine selectively affects D_1 dopamine receptor-mediated activation of striatal Gs proteins. J Pharmacol Exp Ther 1995;273:492–8.

Wang XH, Levitt P, O'Brien JA, Murphy EH. Normal development of tyrosine hydroxylase and serotonin immunoreactive fibers innervating anterior cingulated cortex and visual cortex in rabbits exposed prenatally to cocaine. Brain Res 1996;715:221–4.

Weaver DR, Roca AL, Reppert SM. c-*fos* and *jun*-B mRNAs are transiently expressed in fetal rodent suprachiasmatic nucleus following dopaminergic stimulation. Dev Brain Res 1995;85:293–7.

Weise-Meyer DE, Silvestri JM, Lin D, Buhrfiend CM, Lo ES, Carvey PM. Effect of cocaine in early gestation on striatal dopamine and neurotrophic activity. Pediatr Res 1993;34:389–92.

Woolverton WL, Johnson KM. Neurobiology of cocaine abuse. Trends Pharmacol Sci 1992;13:193–200.

Xu M, Hu XT, Cooper DC, Moratalla R, Graybiel AM, White FJ, Tonegawa S. Elimination of cocaine-induced hyperactivity and dopamine-mediated neurophysiological effects in dopamine D_1 receptor mutant mice. Cell 1994;79:945–55.

Young ST, Porrino LJ, Ladarola MJ. Cocaine induces striatal c-*fos* immunoreactive proteins via dopaminergic D_1 receptors. Proc Natl Acad Sci USA 1991;88:1291–5.

Zuckerman B, Frank DA, Hingson R, Amaro H, Levenson SM, Kayne H. Effects of maternal marijuana and cocaine use on fetal growth. New Engl J Med 1989;12:762–8.