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Prenatal Cocaine Exposure Revealed Minimal Postnatal Changes in Rat Striatal Dopamine D_2 Receptor Sites and mRNA Levels in the Offspring

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Abstract

It has been reported from this laboratory that prenatal cocaine exposure results in the postnatal transient alterations of rat striatal dopamine uptake sites examined from postnatal 0–32 wk. The present study aims to examine whether this will result in a direct/indirect stimulation of dopamine D_2 receptors. Pregnant rats were dosed orally with cocaine hydrochloride (60 mg/kg/d) from gestational day (GD) 7–21. Control animals received an equivalent volume of water. The striatum from the offspring at postnatal 0–32 wk was examined. The radioligand [^{3}H]sulpiride was used for the Scatchard analysis of the D_2 receptors, and the changes in the levels of mRNA for the D_2 receptor were studied using Northern blot analysis. Results from the present study revealed that in the control group, there was an age-dependent increase in the number of D_2 receptor sites (B_{max}: 44.00 ± 2.12 to 178.00 ± 45.10 fmol/mg protein) and in the levels of D_2 mRNA from PN0–32 wk with the most rapid increase occurring during the first 4 wk of postnatal development. Prenatal cocaine exposure resulted in only a significant decrease ($p < 0.001$) in the number of D_2 receptor sites at PN0 wk and in a 10% increase in mRNA levels at PN3, 4, and 12 wk. It was concluded from this study that prenatal cocaine exposure resulted in minimal postnatal changes in the dopamine D_2 receptor.

Index Entries: Cocaine; prenatal; dopamine D_2 receptor, [^{3}H]sulpiride; striatum; rat.

Introduction

In the past decade, there has been a growing health problem owing to the increase in cocaine use among pregnant women (Chasnoff et al., 1987, 1990; Mofenson and Caraccio, 1987; Frank et al., 1988; Little et al., 1988; O'Malley et al., 1988; Neerhof et al., 1989). Clinical studies

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have shown that infants born to cocaine-abusing mothers had altered behavioral patterns characterized by abnormal sleep patterns, poor feeding, tremors and hypertonia (Oro and Dixon, 1987), underarousal (Corwin et al., 1992) and hyperresponsiveness (Freier et al., 1991), impairment in orientation, motor ability, and state regulation behaviors, as well as abnormal reflexes (Chasnoff et al., 1985, 1986, 1989). However, contrary to these reports, there are studies revealing that cocaine-exposed infants had little (Neuspiel et al., 1991) or no neurobehavioral dysfunction (Schneider et al., 1989; Eisen et al., 1991; Richardson and Day 1991; Coles et al., 1992).

Animal studies of prenatal cocaine exposure also provided evidence of postnatal age dependent behavioral changes, such as an increase or decrease in locomotor activity (Hutchings et al., 1989; Smith et al., 1989; Spear et al., 1989; Church and Overbeck, 1990; Henderson and McMillen, 1990; Church et al., 1991; Johns et al., 1992a; Peris et al., 1992), hyperresponsiveness to stress (Smith et al., 1989; Spear et al., 1989; McMillen et al., 1991; Bilitzke and Church, 1992; Johns et al., 1992b), delayed or accelerated acquisition of the righting reflex (Henderson and McMillen, 1990; Sobrian et al., 1990), and accelerated development in acoustic startle response (Davis, 1985; Sobrian et al., 1990). Fetal behavioral changes were also observed (Burchfield et al., 1990; Abrams et al., 1992) as shown in the decrease in rapid eye movement and nonrapid eye movement sleep (Abrams et al., 1992). There are, however, as in the human studies, contrary reports to these animal studies revealing little or no behavioral changes in the prenatally cocaine-exposed animals. These studies showed no pronounced changes in exploratory behavior and activity (Riley and Foss, 1991), acoustic startle reflex (Foss and Riley, 1991), conditioned place preference (Heyser et al., 1992), and activity and rest–activity cycle (Zmitrovich et al., 1992).

Despite the inconsistency of the reports on the behavioral outcome of prenatal cocaine exposure, it is, however, known that cocaine has several sites of action in the adult central nervous system. Cocaine has been shown to block the reuptake of dopamine (DA), norepinephrine (NE), and serotonin (5-HT) (*see* Carroll et al., 1992), and the cocaine binding site on the DA transporter has been implicated in the reinforcing and addicting properties of cocaine (Ritz et al., 1987; Kuhar et al., 1991). Since the mesocorticolimbic and nigrostriatal dopaminergic system has been shown to be involved in behavior, such as reinforcement, arousal, environmental challenges, sensorimotor integration, and perception of stress (*see* Le Moal and Simon, 1991 for review), the influence of prenatal cocaine exposure on the dopaminergic system of the offspring has received some attention.

A number of animal studies on prenatal cocaine exposure have shown changes in the DA receptors, although the findings have been inconsistent. It had been shown autoradiographically that at postnatal days (PND) 11 and 20, there is an increase in D_1 DA receptor binding in the striatum (Dow-Edwards, 1989) as well as in the substantia nigra at PND60 of the cocaine-exposed offspring (Dow-Edwards et al., 1990). This change in D_1 DA receptor was, however, not observed at PND21 in the striatum and nucleus accumbens using Scatchard analysis (Scalzo et al., 1990). Changes in the D_2 DA receptor include a decrease in the K_d of the D_2 DA receptor in the striatum at PND21 (Scalzo et al., 1990) and a decrease in B_{max} in the striatum at PND180 (Henderson et al., 1991). However, contrary to this, no change in D_2 DA receptor binding or the rate of DA turnover in the striatum was observed at PND14 (Fung et al., 1989). Other changes observed in the dopaminergic system also include the decrease in the number of electrophysiologically measured spontaneously active DA cells in the substantia nigra and ventral tegmental area at PND56–68 (Minabe et al., 1992). Elevated whole-brain tyrosine hydroxylase activity measured at birth was also observed in cocaine-exposed rats (Meyer and Dupont, 1993). The discrepancies in the results observed may be because of the differences in drug dosage or treatment regimen, or the method of

analysis. The differences in the age of the subject studied could be a crucial factor that makes interpretation difficult, since this may undermine any transient effects of cocaine not observed in a fixed time-point study. Recently, it was shown in a long-term study from postnatal (PN) 0–32 wk that there is a transient decrease in the number of [^{3}H]mazindol-labeled DA uptake sites in the rat striatum at PN3 and 4 wk in the cocaine-exposed offspring (Stadlin et al., 1994). It had been suggested that blocking of DA uptake results in an indirect stimulation of DA receptors (Peris and Zahniser, 1989). Also, a previous report on MPTP-induced hemiparkinsonism showed that a decrease in [^{3}H]mazindol-labeled DA uptake sites was accompanied by the increase in [^{3}H]spiperone binding of D_2 receptors (Joyce et al., 1986). In light of this, the present study aims to observe further changes in the D_2 DA receptors over the period of PN0–32 wk in order to ascertain whether similar transient effects could also be observed. Scatchard analysis were used to measure the B_{max} and K_d, and Northern blot analysis was used to measure the changes in mRNA levels in the DA D_2 receptor.

Materials and Methods

Animal Treatment

Twenty-five female Sprague-Dawley rats (supplied by the animal house of the Medical Faculty, The Chinese University of Hong Kong) were used in this experiment. Animals were housed in stainless-steel cages on a 12-h light/dark cycle with free access to food and water. The animals were mated with males of the same strain, and the morning when a sperm plug was found was designated gestational day (GD) 1. The pregnant dams were randomly assigned to two treatment groups: cocaine-exposed and control rats. On GD 7–21, the pregnant rats were dosed orally at 9:00–9:30 AM each day with 60 mg/kg/d cocaine hydrochloride (Sigma Chemical Co., St. Louis, MO) for the cocaine-exposed group, and the control animals received an equivalent volume of water; 60 mg/kg/d of cocaine was chosen for this experiment because results from previous studies using cocaine doses ranging from 40–90 mg/kg/d showed an increase in fetal anomalies if the prenatal dose was greater than this (Gingras et al., 1992).

The day of delivery is designated PN d/wk 0. The dams were weighed before delivery, and the meal maternal weight gain was measured as the difference in weight at GD 7 and before delivery. Following delivery, the pups were weighed and each litter was culled to 8–10 pups, keeping to a sex ratio as equal as possible. Pups from the cocaine group were not fostered to nontreated dams; their mothers did not receive any further cocaine treatment after the day of delivery. Crossfostering was not used in order to approximate the human condition. Furthermore, the plasma half-life of cocaine in the rat and human is in the range of 40–80 min (Wiggins, 1992), which would produce minimal residual effects and thus would be expected to be rapidly cleared from the mother's body. Pups that were used for binding assay studies beyond 28 d of age were weaned at PND28 and housed in separate cages until the time period required for the binding assay studies.

Binding Activity of the Dopamine D_2 Receptor

On PN0, 1, 2, 3, 4, 12, and 32 wk, for each treatment group, 2–17 animals were selected at random and sacrificed by decapitation. The number of animals sacrificed depended on the quantity of tissue needed for the binding assays. The binding assay for each time-course and each treatment was done in triplicate. The binding assays were repeated three times, the offspring of each repeated assay were from different pregnant dams. The total number of brains used for binding assays at PN0, 1, 2, 3, 4, 12 and 32 wk for each of the control and cocaine-treated groups were 52, 28, 20, 18, 16, 10, and 6, respectively. A total of 300 pups for the two treatment groups were used for the

binding studies. The striatum from each animal were studied for each time-course.

[^{3}H]sulpiride (65.2 Ci/mmol; Dupont-New England Nuclear, Boston, MA) was used to determine D_2 receptor binding. The striatum studied were removed and dissected on ice. Brain samples from each region were weighed, pooled, and homogenized with 40 vol of ice-cold 50 m*M* Tris-HCl buffer with 120 m*M* NaCl and 5 m*M* *K*Cl (pH 7.4) in a glass-Teflon™ homogenizer. The homogenate was centrifuged at 50,000*g* for 10 min, and the resulting pellets washed twice. The final pellets were rapidly frozen on dry ice and stored at –70°C until assayed, usually within 2 wk of initial homogenization. On the day of binding assay, samples were thawed at 4°C and resuspended in 10 vol of 50 m*M* Tris-HCl buffer (as above) for 30 min; 50-µL aliquots of tissue sample (protein concentration of 1.0 mg/mL) were incubated in triplicate in a total volume of 500 µL. After 30 min of incubation at 4°C with [^{3}H]sulpiride (0.156–20 n*M* for Scatchard analysis, 10 n*M* for single point assays), the samples were filtered rapidly through Whatman GF/B filters with a Brandel Cell Harvester followed by washing of the filters (3 × 5 mL Tris-HCl buffer at 4°C); 10 µ*M* of sulpiride (Research Biochemicals Incorporated, Natick, MA) were used to define specific binding for D_2 receptors. The filters were immersed into 3 mL of scintillant and extracted overnight; the radioactivity was quantitated in a Beckman LS1801 liquid scintillation counter at 48% efficiency. Aliquots of membrane preparations were used for the determination of protein content by the method of Lowry et al. (1951) with bovine serum albumin as the standard.

Estimates of the equilibrium dissociation constant (K_d) and the number of binding sites (B_{max}) were determined using the nonlinear computer curve fitting program LIGAND (Munson and Rodbard, 1980). Statistical differences were tested at each specific age between the control and cocaine-exposed groups using an unpaired Student's *t*-test. Significance was assumed at the level of $p < 0.05$. Results are expressed as mean ± SEM.

Northern Blot Analysis of D_2 Receptor

Poly (A)+ RNA was purified from rat striatum at each age group studied (PN0, 1, 2, 3, 4, 12, and 32 wk) using Fast Track mRNA purification columns (Invitrogen, San Diego, CA); 3 µg poly (A)+ RNA/lane were electrophoresed in a 1% formaldehyde-agarose gel according to Sambrook et al. (1989) and blotted onto a BA-S nitrocellulose membrane (Schleicher and Schuell). Prehybridization was done for 4 h at 42°C in a buffer containing 50% formamide, 5X SSC (0.15*M* NaCl, 0.015*M* sodium citrate, pH 7.2), 5X Denhardt's reagent, 0.5% SDS, and 0.1 mg/mL salmon sperm DNA. The hybridization buffer consisted of prehybridization buffer with 10% dextran sulfate and 5×10^6 cpm/mL of ^{32}P-labeled DNA probe. The rat cDNA probe (approx 2.9 kb) that was the coding region of D_2 receptor fragment (Bunzow et al., 1988) was isolated by PCR, and the whole-length rat cDNA probe of the β-actin (approx 2.5 kb), which had an SA of 10^9 cpm/µg of DNA, was used. After overnight hybridization at 42°C, the blot was washed twice in 1X SSC and 0.1% SDS for 30 min at room temperature. The final two washes were 30 min in 1X SSC and 0.1% SDS at 65°C. A RNA ladder (BRL) was used to standardize the RNA sizes. The bands were quantitated using a densitometer (Molecular Dynamics), and the percentage of change between the control and the cocaine-exposed group was measured as the ratio of D_2 receptor/β-actin labeled. Striatum from a total 288 animals at all age groups studied were removed for the Northern blot analysis. The Northern blot analysis was repeated three times.

Results

Table 1 shows that there was no significant difference between the control and cocaine-exposed group in mean litter size, the mean maternal weight gain during GD 7–21 (before term), and the mean birth weight of the offspring. There was also no difference in the

Table 1
Effects of Cocaine Exposure on Litter Size, Mean Birth Weight, and Maternal Weight Gain During GD 7–21 (Before Term)[a]

	Control	Cocaine-exposed
Litter size	12.0 ± 2.4	13.0 ± 1.4
Mean maternal weight gain before term (g)	149.7 ± 7.0	146.3 ± 5.2
Mean birth weight (g)	6.8 ± 0.7	7.0 ± 0.5

[a]Value ± SEM.

Table 2
[^{3}H]Sulpiride Binding (B_{max} and K_d) to Striatal Membrane in Control and Cocaine-Exposed Rat Offspring[a]

	B_{max}, fmol/mg protein		K_d, nM	
Wk	Control	Cocaine-exposed	Control	Cocaine-exposed
0	44.00 ± 2.12	29.55 ± 2.05[a]	15.23 ± 1.84	9.97 ± 1.53
1	35.70 ± 5.09	34.03 ± 0.81	6.29 ± 0.28	5.82 ± 0.61
2	56.43 ± 1.63	72.33 ± 8.88	3.52 ± 0.32	3.68 ± 0.11
3	98.00 ± 22.63	105.50 ± 19.09	3.77 ± 0.70	3.36 ± 0.08
4	133.90 ± 39.11	130.03 ± 21.49	3.53 ± 0.93	2.93 ± 0.38
12	144.13 ± 42.20	124.78 ± 10.53	4.24 ± 1.07	4.49 ± 1.51
32	178.00 ± 45.10	159.00 ± 28.80	2.77 ± 1.01	2.93 ± 0.72

[a]A significant decrease in B_{max} was found in cocaine-treated offspring at PN0 wk. Membranes were incubated with [^{3}H]sulpiride (1.56–20 n*M*) with or without 10 μ*M* of sulpiride. $N = 9$ at each time-point. Measurement was in triplicate with each experiment repeated three times independently. *$p < 0.001$ significant difference from the water control.

number of stillbirths and deformities observed between the two groups (observation not shown). The specific binding of [^{3}H]sulpiride in the control group at PN0 wk is only 58%. There is an age-dependent increase to 86% by PN4 wk.

Table 2 shows the Scatchard analysis of [^{3}H]sulpiride-labeled D_2 receptor. There is an age-dependent increase in the number of D_2 receptor sites (B_{max}) from PN0–32 wk (44.00 ± 2.12–178.00 ± 45.10 fmol/mg protein) in the control group. The increase in B_{max} was rapid during the first 4 wk of development, having a threefold increase at PN4 wk when compared to PN0 wk. This is followed by a slight increase until PN32 wk. In the cocaine-exposed group, there is a significant decrease ($p < 0.001$) in the number of receptor sites at PN0 wk. However, there is no significant difference in the number of D_2 receptor sites from PN12–32 wk between the control and the cocaine-exposed group, although there is a trend of increase in the number of receptor sites at PN2 wk in the cocaine-exposed group. At PN12 and 32 wk, there is a slight trend of decrease in the number of receptor sites in the cocaine-exposed group.

The dissociation constant (K_d) at PN0 is 15.23 ± 1.84 n*M*. This is three times higher than that of PN2–32 wk. However, there is no significant difference in K_d values between the control and cocaine-exposed group from PN0–32 wk (Table 2).

The control group showed an age-dependent increase in the levels of D_2 mRNA labeling with a 1.8-fold increase in density levels at PN32 wk when compared to PN0 wk (Fig. 1).

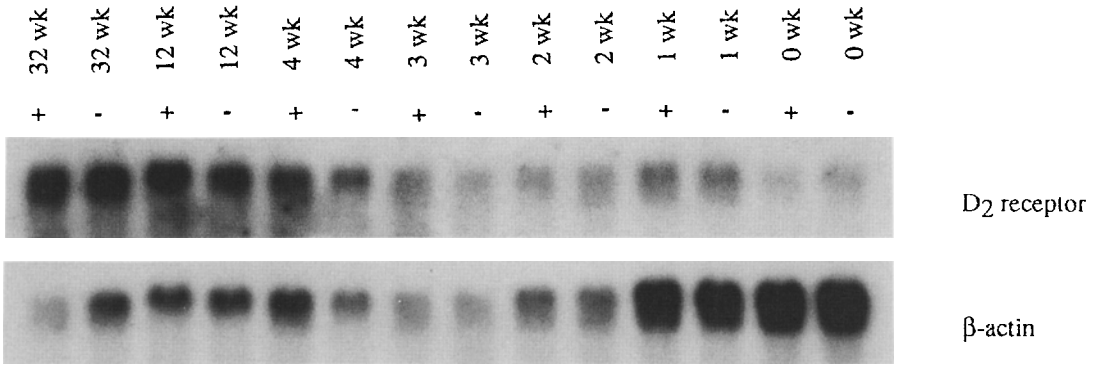

Fig. 1. A representative autoradiograph from Northern blots of the D_2 receptor cDNA probes following hybridization with mRNA from isolated striatal tissue. (–) represents blots from the control group and (+) represents blots from the cocaine-exposed group. D_2 receptor cDNA probe is ~2.9 kb and β-actin cDNA probe is ~2.5 kb. There is an age-dependent increase in the levels of D_2 receptor mRNA labeling in both groups.

The cocaine-exposed group showed an approx 10% increase in density levels at PN3, 4, and 12 wk when compared to that of the control group (Fig. 2).

Discussion

Both Scatchard analysis and Northern blot analysis on the D_2 DA receptor showed an age-dependent increase in D_2 receptor number and mRNA synthesis during PN0–32 wk with the most rapid increase occurring during the first 4 wk of development. This trend in the development of the D_2 receptor was similar to that observed by Nomura et al. (1982) using [^{3}H]spiperone binding, where a rapid increase in D_2 receptor sites, tripling and reaching a peak by PN4 wk, was also observed.

In this study, the exposure of maternal cocaine at 60 mg/kg/d did not seem to affect the litter size, offspring birth weight, and maternal weight gain during GD 7–21. Therefore, any changes reported may not be the result of nutritional factors.

From the present results, the control group showed a single binding site for [^{3}H]sulpiride with a B_{max} ranging from 44–178 fmol/mg protein and a K_d ranging from 15.23–2.77 nM from PN0–32 wk. The B_{max} and K_d (with the exception of PN0 wk) values reported in this study are in agreement with previous published data on adult rat striatum B_{max} values of 240 fmol/mg protein (Freedman et al., 1981) and K_d values of 3.2 nM (Jastrow et al., 1984), respectively. Of all the age groups studied, only animals at PN0 wk showed a significant difference in B_{max} value. The value for the specific binding of [^{3}H]sulpiride at PN0 is only 58%. Therefore, the B_{max} and K_d values may not be entirely reliable. Another factor for this difference between the control and cocaine-exposed group could be the residual effects of maternal cocaine, since the pups were not fostered to untreated dams. Northern blot analysis, however, revealed no difference in the mRNA levels between the two groups at PN0, further suggesting that the difference observed may be the result of these abovementioned factors.

Scatchard analysis revealed no significant changes in the B_{max} and K_d of the D_2 receptor, although a trend in the increase in B_{max} at PN2 wk was observed. This is partially in agreement with that observed by Scalzo et al. (1990), where an approx 17% increase in receptor binding was observed in the cocaine-exposed group when measured at PN3 wk. At PN12 and 32 wk, there seemed to be a reversal of trend where an approx 10–13% decrease in B_{max} was observed. This reversal of trend was also reported by Henderson et al. (1991), showing a 23%

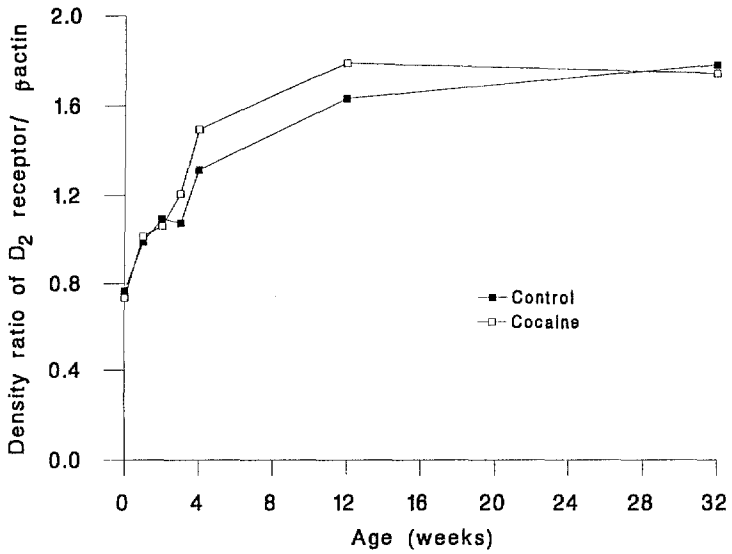

Fig. 2. Relative levels of D2/β-actin mRNA were compared between the control and cocaine-exposed groups. There is an age-dependent increase in the density levels in both groups with the cocaine-exposed group showing a 10% decrease in density levels at PN3, 4, and 12 wk. Values are means of density ratio levels from three Northern blot analyses.

decrease in B_{max} by PN25–26 wk after an initial slight increase (not significant) at PN4 wk in the cocaine-exposed group.

Northern blot analysis showed a 10% increase in mRNA levels at PN3, 4, and 12 wk. This corresponded to the same period where a 10% decrease in D_1 receptor binding was observed in the cocaine-exposed group (unpublished data). However, this did not have the same magnitude of change as observed in the previous study, where a 39 and 21% decrease in DA uptake sites at PN3 and 4 wk, respectively, was reported (Stadlin et al., 1994). This suggested that despite the changes in the DA uptake sites, there were minimal changes in the DA D_2 receptor sensitivity in the striatum. This response of D_2 receptor is different to that reported in nigrostriatal denervation studies, where a 40% (Graham et al., 1990) and 100% (Joyce et al., 1986) increase in D_2 receptors were observed accompanying a loss of over 90% of DA uptake sites.

In conclusion, the results from the present study seem to suggest that prenatal cocaine exposure, resulting in the presynaptic changes of neurotransmitter reuptake, the main site of action of cocaine, is not reflected by concomitant changes in receptor sensitivity.

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