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RAPID COMMUNICATION

Weanling Rats Exposed Prenatally to Cocaine Exhibit an Increase in Striatal D2 Dopamine Binding Associated With an Increase in Ligand Affinity

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SCALZO, F. M., S. F. ALI, N. A. FRAMBES AND L. P. SPEAR. *Weanling rats exposed prenatally to cocaine exhibit an increase in striatal D2 dopamine binding associated with an increase in ligand affinity.* PHARMACOL BIOCHEM BEHAV **37**(2) 371–373, 1990.—Prenatal exposure to cocaine can result in abnormal neurobehavioral development. This study found an incease in D2 dopamine receptor binding, associated with an increase in ligand affinity, in striatum of weanling rats exposed prenatally to cocaine. There were no changes in D2 receptor binding in nucleus accumbens nor D1 receptor binding in either striatum or nucleus accumbens. Alterations in D2 dopamine receptors may be associated with neurobehavioral alterations following prenatal cocaine exposure.

Cocaine Prenatal D2 receptor Striatum

OVER the past decade, there has been a substantial increase in the population of human infants whose mothers have used cocaine during pregnancy (2, 10, 16). Both clinical investigations (3–6) and studies in laboratory animals (14, 24–26) have reported altered behavioral/cognitive function in offspring exposed early in life to cocaine. A number of researchers are beginning to examine the profile of neural alterations resulting from such cocaine exposure.

Available evidence suggests that chronic treatment with the dopamine (DA) uptake inhibitor cocaine during ontogeny may result in subsequent alterations in the DA system later in life. For instance, administration of cocaine during the early postnatal period has been reported to increase glucose metabolism and D1 dopamine receptor binding in a number of dopaminergic brain regions, with these effects being dependent upon the sex of the animal and the age of postnatal treatment (8, 9, 23). Gestational cocaine exposure has been reported to alter later psychopharmacological and neuropharmacological sensitivity to dopaminergic manipulations (26,27), suggesting that alterations in the DA system may also result from in utero cocaine exposure.

The present study was designed to examine D1 and D2 dopamine receptor binding characteristics, using the specific ligands [^{3}H]SCH-23390 and [^{3}H]spiroperidol, respectively, in membranes prepared from striatal and nucleus accumbens tissue of weanling offspring exposed gestationally to cocaine. Examination of D1 binding characteristics were conducted in these offspring to determine whether gestational cocaine exposure would result in an increase in D1 binding as has been recently reported for cocaine treatment during the early postnatal period (23).

METHOD

Gravid Sprague-Dawley rats were placed into one of two treatment groups: one which received subcutaneous (SC) injections of 40 mg/kg/3 cc cocaine hydrochloride daily from gestational days 8–20, and another which received SC injections of 0.9% saline over the same gestation period [see (26) for dosing rationale]. On the day after birth, postnatal day (PND) 1, each litter was culled to 8–10 pups per litter and fostered to an untreated surrogate dam where they were left undisturbed until PND 21. On PND 21, four male pups per litter were sacrificed and their brains rapidly removed and placed on ice. Striatum and nucleus accumbens were quickly removed by free-hand dissection (13), weighed,

TABLE 1

D1 AND D2 DOPAMINE RECEPTOR BINDING IN NUCLEUS ACCUMBENS AND STRIATUM (MEAN fmoles/mg PROTEIN ± S.E.M.) OF OFFSPRING EXPOSED TO SALINE OR 40 mg/kg COCAINE ON GESTATIONAL DAYS 8–20

	Striatum		Nucleus Accumbens	
Prenatal Treatment	D1	D2	D1	D2
Saline Control	365.3	139.0	142.5	80.3
N=8	±26.3	±7.9	±18.7	±6.0
Cocaine (40 mg/kg)	370.8	163.6*	150.3	82.4
N=8	±9.8	±7.9	±17.9	±7.5

A significant increase in striatal D2 binding was found in cocaine-treated offspring. Membranes from nucleus accumbens and striatum were incubated with either 1.0 nM [^{3}H]SCH-23390 or [^{3}H]spiroperidol with or without 1 μM (+)butaclamol. N=number of rats/group. *$p<0.05$, significantly different from saline controls.

frozen on dry ice and stored at −70°C until time of assay. Litter was considered to be the unit of analysis, with pups from eight litters being represented in each treatment group.

Membranes for receptor binding assays were prepared from striatal and nucleus accumbens tissue as previously described (1). D1 and D2 dopamine receptor binding was assayed using aliquots of membrane preparations incubated with [^{3}H]SCH-23390 (81.0 Ci/mmol, New England Nuclear, Boston, MA), for D1, or [^{3}H]spiroperidol (24.2 Ci/mmol; New England Nuclear, Boston, MA), for D2 binding. Single point assays were performed on aliquots of striatal or nucleus accumbens membranes following methods previously described (1) at a concentration of 1.0 nM for each ligand and 1.0 μM unlabelled competitor. Scatchard analysis was then conducted on D1 and D2 binding in striatal membranes using the same binding method and ligand concentration of 0.02–2.00 nM. Incubations were carried out in triplicate for 20 min at 37°C in a total volume of 1 ml. Parallel incubations were performed in the presence of 1 μM (+)butaclamol (Research Biochemical, Inc., Wayland, MA). Total radioactivity was quantified by liquid scintillation spectrometry (Tracor Mark III, Elk Grove Village, IL). Specific binding was calculated as the difference between the amount of [^{3}H]SCH-23390 or [^{3}H]spiroperidol binding alone (total binding) and that in the presence of 1.0 μM (+)butaclamol (nonspecific binding). Aliquots of membrane preparations were used for the determination of protein content by the method of Lowry (17) using bovine serum albumin (Sigma Chemical Co., St. Louis) as the standard. Estimation of the equilibrium dissociation constant (K_d) and the number of binding sites (B_{max}) was performed by Scatchard analysis using a linear regression analysis (22). Statistical differences were tested using Student's *t*-test.

RESULTS

The single point studies revealed an increase in [^{3}H]spiroperidol binding in the striatum of animals exposed to 40 mg/kg cocaine during gestation, $t(14)=2.20$, $p<0.05$ (Table 1). There was no difference between treatment groups in nucleus accumbens [^{3}H]spiroperidol binding. There were also no differences between treatment groups in [^{3}H]SCH-23390 binding for either brain region (Table 1). Scatchard analysis of D1 and D2 receptor binding in the striatum showed that there was a decrease in K_d in cocaine-treated animals compared to saline-treated controls, $t(6)=3.47$, $p>0.05$. This change in affinity was not accompanied by a change in B_{max} (Table 2).

DISCUSSION

In this study, D2 binding was observed to be increased in striatum following gestational cocaine exposure. No alterations in D1 binding were observed following gestational cocaine exposure in membranes from either nucleus accumbens or striatum. This lack of alteration in D1 binding is in contrast to the increases in D1 binding reported in the nucleus accumbens of males and the caudate of females in autoradiograms of adult offspring following cocaine administration from 11–20 days postnatally by Segal *et al.* (23). Such increases were not seen with cocaine treatment during the 1–10-day postnatal period (23), suggesting the possibility that D1 receptor upregulation may only be evident following chronic cocaine treatment relatively late in ontogeny.

Scatchard analysis revealed that the increase in D2 binding in striatum following gestational cocaine treatment was the result of an increase in affinity rather than an increase in the number of binding sites. This is a rather surprising finding in that often the receptor alterations following drug treatment either during development or in adulthood are associated with alterations in B_{max} rather then K_d. For instance, alterations in DA binding observed after pre- or early postnatal treatment with DA antagonists are related to changes in the number of receptors with no change in affinity (15, 19, 21). However, alterations in K_d for DA receptors have been reported in a number of instances following pharmacological or dietary manipulations early in life, including neonatal 6-hydroxydopamine treatment {[^{3}H]domperidone binding; (7)}, dietary lithium {[^{3}H]spiperone binding; (28)}, and lead exposure {[^{3}H]haloperidol; (20)}. What factors influence whether alterations in receptor affinity versus number will be seen following a given challenge early in life still remain to be determined.

The increase in D2 binding in striatum following gestational

TABLE 2

B_{max} (fmoles/mg PROTEIN) AND K_d (nM) FOR D1 AND D2 DOPAMINE RECEPTORS IN STRIATUM OF WEANLING OFFSPRING EXPOSED TO SALINE OR 40 mg/kg COCAINE ON GESTATIONAL DAYS 8–20

	D1 [^{3}H]SCH-23390		D2 [^{3}H]Spiroperidol	
Prenatal Treatment	B_{max}	K_d	B_{max}	K_d
Saline Control	366.7 ± 17.8	1.22 ± 0.09	233.5 ± 22.5	0.62 ± 0.01
Cocaine (40 mg/kg)	337.4 ± 15.8	1.27 ± 0.04	208.9 ± 18.2	0.54 ± 0.02*

A significant reduction in the K_d of D2 receptors was found in cocaine exposed offspring. Each B_{max} ± S.E.M. and K_d ± S.E.M. is the mean of four experiments, each of which represents tissue pooled from two litters. *$p<0.05$, significantly different from saline controls.

cocaine exposure in this experiment is reminiscent of the findings of Rosengarten and Friedhoff (19) where gestational exposure to the indirect DA agonist L-DOPA was observed to result in an increase in [^{3}H]spiroperidol labeled DA binding sites. However, recently Fung and associates (12) have reported that chronic exposure to 30 mg/kg/day cocaine throughout gestation administered via SC osmotic minipumps produced no alterations in the B_{max} or K_d of [^{3}H]spiperone binding in striatal tissue of 14-day-old offspring. There are marked differences in the physiological response to intermittent injections versus continuous minipump exposure in adulthood with, for instance, sensitization being observed with intermittent cocaine exposure and tolerance observed with continuous cocaine exposure [e.g., (18)]. Thus, it is perhaps not unexpected that the effects of intermittent versus continuous cocaine exposure during gestation may also vary. To the extent that animal studies are designed to model human use patterns, intermittent drug administration schedules may prove to be more appropriate.

Alterations observed in D2 binding after early pharmacological challenges, such as the increases in D2 binding observed following gestational cocaine in the present study, may be related to drug-induced alterations in endogenous ligand/receptor interactions as these receptors are maturing [see (11) for further discussion]. Alternatively, these receptor modifications may not be solely a consequence of drug-induced alterations in the amount of endogenous DA reaching these nascent receptors, but may instead reflect compensatory processes occurring in response to effects of the drug challenge on the development of other aspects of the DA system (e.g., alterations in terminal proliferation). Further work is needed to characterize the potentially complex profile of DA alterations induced by gestational cocaine exposure and to determine the impact of these alterations on neurobehavioral function.

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