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Neurochemical Effects of Prenatal Haloperidol Exposure

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SCALZO, F. M., R. R. HOLSON, B. J. GOUGH AND S. F. ALI. *Neurochemical effects of prenatal haloperidol exposure.* PHARMACOL BIOCHEM BEHAV **34**(4) 721–725, 1989.—The neurochemical effects of prenatal exposure to dopamine receptor antagonists are as yet poorly characterized. To further examine this problem, pregnant rats were given daily subcutaneous injections of vehicle, 2.5 or 5.0 mg/kg haloperidol over gestational days 6 through 20. Membrane binding of [^{3}H]SCH-23390 (D1-specific) and [^{3}H]spiroperidol (D2-specific in most brain areas) was measured in four regions of the cerebral dopamine system at postnatal day (PND) 30. Dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC) levels were measured in caudate on PND 30 following a d-amphetamine challenge. Prenatal haloperidol exposure reduced [^{3}H]SCH-23390 and [^{3}H]spiroperidol binding in caudate in a dose-dependent manner. [^{3}H]Spiroperidol binding was similarly reduced in nucleus accumbens, but only the low dose (2.5 mg/kg) group showed decreased [^{3}H]SCH-23390 binding in this region. Binding of neither compound was significantly altered in amygdala or frontal cortex. Basal or drug-stimulated levels of caudate DA and DOPAC were unaltered. It is concluded that prenatal haloperidol exposure reduces D1 and D2 binding in some, but not all regions of the forebrain dopamine system.

Dopamine receptors Development Haloperidol Prenatal drug effects

THE study of the effects of chronic neuroleptic exposure on the dopamine (DA) system during development is still very much in its infancy. Currently, reports suggest that prenatal exposure to neuroleptics in rats causes a long-lasting reduction in caudate D2, but perhaps not caudate D1 binding sites (1, 3, 5, 12, 14). This finding is not without controversy, however. A carefully conducted study by Madsen and colleagues (9) did not replicate this effect on caudate binding. The authors suggest that early effects of such exposure may be attributable to a lingering direct action of haloperidol (HAL), which as they showed is cleared very slowly from the neonatal brain. Even more puzzling, Moon (11) has reported that administration of chronic HAL by osmotic minipump over the course of gestation caused an apparent increase in [^{3}H]spiroperidol binding in the caudate nucleus of exposed offspring.

The issue of the impact of early neuroleptic administration upon neurotransmitter levels in the developing dopamine system is even more clouded. Rosengarten and Friedhoff (13) found no effect of prenatal HAL exposure upon basal or HAL-stimulated levels of DA, 3,4-dihydroxyphenylacetic acid (DOPAC) or homovanillic acid (HVA) in the caudate. However, in studies where HAL exposure was extended to include the early postnatal as well as prenatal period, an enduring decrease was seen in dopamine levels in the caudate (7).

The extremely sketchy and often controversial nature of reported neurochemical effects of early exposure to neuroleptics underlines the necessity of improving our understanding of this important problem. Clearly, the discrepancies in published reports of prenatal HAL effects upon caudate dopamine receptors require resolution. Even more, study of this problem must widen beyond the current almost exclusive focus upon the caudate, to include additional regions of the dopamine system. In this context, it is also important to utilize recent improvements in ligand specificity to measure drug effects upon both D1 and D2 receptors. Finally, a variety of neurochemical measures of the functional status of the dopamine system must be undertaken, including measurement of basal and challenged levels of major neurotransmitters and metabolites in response to early neuroleptic exposure.

In this paper, we report an initial attempt to address some of the above questions. Effects of prenatal HAL exposure on DA receptors were measured with ligands specific for D1 and D2 sites in the nigrostriatal, mesolimbic and mesocortical subsections of the forebrain dopamine system. Prenatal HAL effects on basal and amphetamine-stimulated levels of caudate DA and DOPAC were also assessed.

METHOD

Subjects

All subjects were from the Sprague-Dawley derived, caesar-

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ean-delivered albino strain maintained at the National Center for Toxicological Research (NCTR). Rats were maintained in a temperature (23°C) and humidity (50%) controlled environment with a 12-hour light/12-hour dark cycle (lights on at 0700 hours). Cage bedding was changed twice a week throughout gestation and weaning. Food and water were available ad lib. Nulliparous females were exposed to experienced male breeders overnight when they were approximately 10 weeks old. The next morning (gestational day 0), all plug-positive females were housed individually. Maternal weights were collected daily from onset of dosing to parturition. At parturition (postnatal day 1), litters were weighed, sexed and randomly culled to 4 ± 1 pups of each sex. Pups remained with the mother until weaning into same-sex group caging at postnatal day (PND) 21. Body weights of offspring were recorded on PNDs 1, 30 and 62, prior to testing. All neurochemical analyses were conducted on offspring sacrificed at PND 29–31.

HAL Exposure

HAL (Sigma Chemical, St. Louis, MO) was dissolved in a drop of lactic acid, then brought up to a concentration of 2.5 or 5.0 mg/ml with distilled water. The pH of the final solution was maintained at 4.2 with lactic acid. Solutions were made fresh weekly and kept in light-tight containers. Dams were injected subcutaneously every morning with vehicle (CON), 2.5 (LOW) or 5.0 (HIGH) mg/kg HAL in the vehicle, at a standard volume of 1 ml/kg. Dosing began on gestational day (GD) 6 and continued through GD 20.

Experimental Design and Statistical Analysis

All experiments included subjects of both sexes. For the receptor binding data, brain regions from one male and one female per litter were analyzed with the exception of the nucleus accumbens. For the latter region, an additional independent replicate utilized males and females drawn from separate litters. Consequently, the binding data were analyzed using a mixed-model ANOVA with sex as a within-litters correlated measure [litter (prenatal treatment) × sex], except for nucleus accumbens, where the design was a two-way, sex by prenatal treatment ANOVA. For the PND 30 amphetamine challenge, the number of drug conditions made it impractical to keep sexes paired within litters. Here, too, results were analyzed using sex as an independent factor in a three-way sex by drug challenge by prenatal treatment ANOVA. In all cases, post hoc comparisons were made using Duncan's Multiple Range Test between prenatal treatment groups only if the overall F value was significant.

D1 and D2 Dopamine Receptor Binding

For assay of D1 and D2 binding sites, membranes were prepared from caudates of animals sacrificed on PND 29–31. Animals were sacrificed by decapitation and the brains dissected into caudate, nucleus accumbens, amygdala and frontal cortex according to the methods of Glowinski and Iversen (6). Brain regions were frozen on dry ice and stored at −70°C prior to receptor binding assay using the methods of Ali *et al.* (2).

Tissue was homogenized in 20 volumes (w/v) of 0.32 M sucrose followed by centrifugation (50,000 × g for 10 minutes). The pellet was rehomogenized in deionized distilled water (pH adjusted to 7.4) and centrifuged (50,000 × g for 10 minutes). The pellet was then resuspended in 50 mM Tris-HCl (pH 7.4) buffer and centrifuged (50,000 × g for 10 minutes). The final pellet was resuspended in the incubation buffer (50 mM Tris-HCl containing 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM KCl, 120 mM NaCl, 0.1% ascorbate and 10 μM pargyline, pH 7.4) at a concentration of 50 mg (original wet weight equivalent/ml). D1 and D2 receptor binding was assayed by incubating 100 μl of the membrane preparation with either 1.0 nM [^{3}H]SCH-23390 (81 Ci/mmol, New England Nuclear, Boston, MA) for D1 binding or 1.0 nM [^{3}H]spiroperidol (24.2 Ci/mmol, New England Nuclear, Boston, MA) for D2 binding. Parallel incubations were performed in the presence of 1.0 μM (+)-butaclamol (Research Biochemical, Inc., Wayland, MA) for both D1 and D2 binding. Incubations were carried out in triplicate for 20 minutes at 37°C in a total volume of 1 ml. After incubation, samples were diluted with 5 ml of ice-cold 50 mM Tris-HCl buffer and rapidly filtered under vacuum through Whatman GF/C glass fiber filters (Whatman, Inc., Clifton, NJ). The filters were washed twice with 5 ml cold Tris-HCl buffer. This separation and washing procedure took less than 20 seconds. The filters were air dried and placed into scintillation vials containing 10 ml of Scintosol® scintillation medium (Isolab, Inc., Akron, OH). Total radioactivity was quantified by liquid scintillation spectrometry (Tracor Mark III, Elk Grove, IL). Specific binding was calculated as the difference between the amount of [^{3}H]SCH-23390 (for D1) or [^{3}H]spiroperidol (for D2) alone (total binding) and that in the presence of 1.0 μM (+)-butaclamol (nonspecific binding). Aliquots of the membrane preparations were used for Lowry protein determinations (8).

Amphetamine Challenge

On PND 30, subjects were injected IP with either 3.5 mg/kg d-amphetamine (Sigma, St. Louis, MO) or saline, and sacrificed one hour later. Animals were sacrificed by decapitation, their brains were removed and quickly dissected on ice into different regions following the methods of Glowinski and Iversen (6), frozen over dry ice and stored at −70°C until analysis. DA and DOPAC were resolved and quantified by HPLC/EC as described by Ali *et al.* (2). Briefly, each caudate was weighed and diluted with a measured volume (10 v/w) of 0.2 N perchloric acid containing 250 ng/ml of the internal standard 3,4-dihydroxybenzylamine (DHBA). Brain tissue was then disrupted by ultrasonication, centrifuged (1,000 × g, 5 minutes) and 150 μl of the supernatant removed and filtered through a 0.2 μm microfilter [MF-1 microcentrifuge filter, Bioanalytic System (BAS), W. Lafayette, IN]. Aliquots of 25 μl representing 10 mg of brain tissue were injected directly onto the HPLC system for separation of DA and DOPAC.

The analytical system included a Waters Associates M-6000A pump (Milford, MA), a Rheodyne® 7125 injector (Rainin Instrument, Woburn, MA), a Biophase® ODS, 5 μ (250 × 4.6 mm) analytical column (BAS), a LC-4A amperometric detector and LC-17 oxidative flow cell consisting of a glassy carbon electrode (TL-5) versus Ag-AgCl reference electrode maintained at a potential of 0.65 V (BAS). The mobile phase consisted of 0.15 M monochloroacetate, pH 3.0, 4.5% acetonitrile and an ion pairing reagent of 0.5 mM octyl sodium sulfate. Chromatograms were recorded and integrated on a 3380A integrator (Hewlett-Packard, Avondale, PA). The endogenous biogenic amine concentrations were calculated using a standard curve for each amine. The standard curves were generated by determining in triplicate the ratio between three different known amounts each amine and a constant amount of DHBA. Concentrations of DA and DOPAC were determined.

RESULTS

Pregnancy Outcome, Maternal and Offspring Body Weights

Prenatal exposure to haloperidol had a substantial effect upon

TABLE 1

PREGNANCY OUTCOMES AND OFFSPRING BODY WEIGHTS FROM FEMALES EXPOSED TO VEHICLE (CONTROL), 2.5 mg/kg HALOPERIDOL (LOW) OR 5.0 mg/kg HALOPERIDOL (HIGH) ON GESTATIONAL DAYS 6–20

Dose	Maternal Weight Gain, GD 6–GD 20 (g)	Gestational Day of Birth	Number Dead Per Litter	Mean Pup Weight at Birth (g)	Weight PND 30 (g) Male	Weight PND 30 (g) Female	Weight PND 62 (g) Male	Weight PND 62 (g) Female
Control	35.5 ± 1.4 (22)	22.1 ± 0.1 (22)	0 (22)	7.3 ± 0.1 (22)	99.0 ± 2.1 (9)	87.3 ± 2.4 (11)	356 ± 11.1 (8)	234 ± 5.3 (8)
Low	22.0 ± 2.1* (16)	22.4 ± 0.2 (16)	0.06 ± 0.1 (16)	6.5 ± 0.1* (16)	82.0 ± 3.7* (7)	82.4 ± 3.2 (6)		
High	14.0 ± 1.5* (15)	22.8 ± 0.1* (15)	0.47 ± 0.2* (15)	6.1 ± 0.2* (15)	84.6 ± 2.6* (9)	76.3 ± 1.3* (9)	321.6 ± 8.3* (8)	222 ± 8.0 (8)

All values are means ± standard error. Number of litters in parentheses.
*Significantly different from controls.
PND—Postnatal Day.

pregnancy outcome and offspring weight (Table 1). Maternal weight gain over the course of exposure showed a dose-dependent decrease [$F(2,50)=46.8$, $p<0.0001$, CON > LOW > HIGH]. HAL also delayed parturition by some 12 hours and increased infant mortality in the HIGH group [Day of birth, $F(2,50)=7.7$, $p<0.005$, HIGH > CON; Dead pups, PND 1, $F(2,50)=4.2$, $p<0.025$, HIGH > CON].

Offspring weight was reduced in both sexes at birth [$F(2,50)=19.9$, $p<0.0001$, HIGH = LOW > CON], and at PND 30, $F(2,24)=11.3$, $p<0.0005$. At PND 62 only CON and HIGH weights were available; at this age HIGH male weight was still 10% lower than controls, $F(1,14)=6.25$, $p<0.05$. PND 62 HIGH female weights were slightly but not significantly lower than CON.

D1 and D2 Receptor Binding

HAL exposure during gestation had a clear effect upon both [^{3}H]SCH-23390 and [^{3}H]spiroperidol binding in some parts of the forebrain dopamine system (Table 2). These effects were seen most clearly in the caudate nucleus, where HAL exposure reduced binding levels of both compounds [D2: $F(2,46)=4.7$, $p<0.01$, CON > HIGH = LOW; D1: $F(2,46)=3.5$, $p<0.05$, CON > HIGH].

Similar effects were seen in D2 binding in the nucleus accumbens. Prenatal HAL exposure resulted in a 20% reduction in [^{3}H]spiroperidol binding, $F(2,66)=5.1$, $p<0.01$. D1 effects were less certain in nucleus accumbens. A significant HAL effect, $F(2,42)=5.5$, $p<0.01$, was due to a large reduction in [^{3}H]SCH-23390 binding in the LOW, but not the HIGH condition. D1 and D2 binding were not altered by prenatal HAL in the other parts of the forebrain dopamine system assayed here. This lack of an effect was particularly clear in the amygdala (Table 2), and for spiroperidol binding in frontal cortex (Table 2).

Neurochemical Response to Amphetamine Challenge

In contrast to the reductions in D1 and D2 binding seen in the caudate nucleus, DA and DOPAC levels seemed unaffected by prenatal HAL treatment (Fig. 1). Amphetamine challenge at PND

TABLE 2

[^{3}H]SCH-23390 (D1) AND [^{3}H]SPIROPERIDOL (D2) BINDING IN VARIOUS REGIONS OF THE FOREBRAIN DOPAMINE SYSTEM IN OFFSPRING OF FEMALES EXPOSED TO VEHICLE (CONTROL), 2.5 mg/kg HALOPERIDOL (LOW) OR 5.0 mg/kg HALOPERIDOL (HIGH) ON GESTATIONAL DAYS 6–20

Brain Region	D1 Control	D1 Low	D1 High	D2 Control	D2 Low	D2 High
Caudate Nucleus	791.8 ± 23.7 (21)	695.9 ± 36.2* (13)	648.9 ± 27.2* (15)	434.1 ± 9.3 (21)	399.4 ± 9.2* (13)	383.7 ± 14.4* (15)
Nucleus Accumbens	34.6 ± 2.9 (16)	20.6 ± 2.7* (10)	28.2 ± 2.2 (22)	180.0 ± 9.9 (26)	140.4 ± 9.4* (16)	148.8 ± 7.9* (30)
Frontal Cortex	4.7 ± 1.1 (7)	8.4 ± 1.7 (8)	9.1 ± 1.9 (9)	144.0 ± 6.3 (7)	149.6 ± 5.2 (8)	148.1 ± 5.9 (9)
Amygdala	36.4 ± 2.5 (7)	34.7 ± 2.1 (8)	34.6 ± 2.2 (9)	70.3 ± 15.4 (7)	51.5 ± 3.7 (8)	57.8 ± 3.6 (9)

All units are means in fmoles/mg protein ± standard errors. Number of subjects or litter means in parentheses.
*Significantly different from control values.

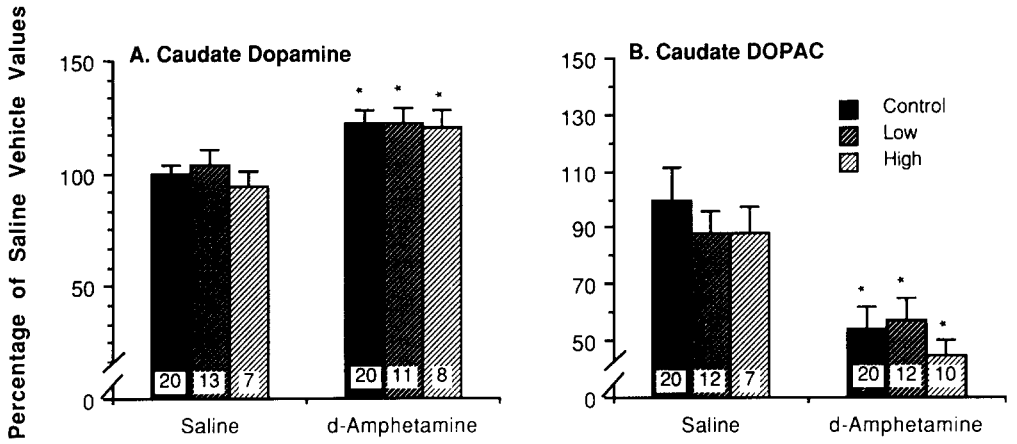

FIG. 1. Neurochemical response to amphetamine challenge. Male and female offspring were given saline or 3.5 mg/kg amphetamine sulfate IP at PND 30 and sacrificed one hour later. Shown are dopamine and DOPAC levels in caudate nucleus (percent of CON values ± standard error of the mean, as a function of prenatal exposure). CON: Prenatal exposure to vehicle. LOW: Prenatal exposure to 2.5 mg/kg HAL. HIGH: Prenatal exposure to 5.0 mg/kg HAL. *Significantly different from saline-injected controls.

30 increased DA and decreased DOPAC content [DA: $F(1,57) = 29.8$, $p<0.0001$; DOPAC: $F(1,57) = 27.7$, $p<0.0001$], but neither basal nor amphetamine challenge levels of these compounds varied as a function of prenatal HAL exposure.

Interaction of Prenatal Treatment With Sex

There was no instance of a significant sex interaction with prenatal HAL in any of the above data (data not show).

DISCUSSION

Of primary importance is the fact that these experiments replicate and extend reports of reduced dopamine receptor binding in brains of animals exposed prenatally to haloperidol. This reduction was especially evident in the caudate nucleus; [^{3}H]spiroperidol (and hence D2) binding was reduced by some 12% in the high-dose litters. This effect, while much smaller than that initially reported by Rosengarten and Friedhoff (12) at the same age, was quite robust. Moreover, we found a 30% reduction in D1 binding in caudate of high-dose prenatal HAL subjects. This more substantial effect was not obtained by Rosengarten *et al.* (14). However, while details differ, it may be concluded that prenatal HAL exposure does indeed reduce D1 and D2 binding in the caudate of exposed offspring on PND 30.

This conclusion evidently extends to the nucleus accumbens, where we obtained a somewhat more substantial D2 reduction (on the order of 20%) in both low and high groups. This reduction may or may not be accompanied, as in the caudate nucleus, by reduced D1 binding. Our finding of a reduction in D1 binding only in the low dose group is puzzling and clearly requires further investigation. There was no evidence for HAL-induced alterations in either D1 or D2 receptors in the amygdala.

These alterations in receptor levels evidently have little direct effect upon dopaminergic neurotransmission as measured in this study. Our finding of a lack of effect of prenatal HAL exposure on basal and amphetamine-stimulated levels of DA and DOPAC at PND 30 replicate a similar report by Rosengarten and Friedhoff (13). A previous report of alterations in these parameters (7) utilized both pre- and postnatal HAL exposure, perhaps indicating that longer-duration exposure is required before one can expect to see such changes.

No prenatal HAL effects reported in this paper interacted with sex, suggesting that sex differences are not important to these drug effects. Surprisingly, prenatal HAL given subcutaneously was toxic to both dams and offspring, in that it reduced maternal and offspring body weight and increased neonatal mortality. This finding contradicts reports of no weight loss in offspring from other labs (9,12) using similar doses, and suggests that in future experiments, pair-fed dams may be an important control.

In closing, the above findings have several implications for the nature and mechanism of prenatal HAL-induced alterations in the dopamine system. First, our failure to obtain similar alterations in all parts of this system certainly indicates that such effects may not be due to the lingering presence of low levels of haloperidol in the CNS, as suggested by Madsen *et al.* (9). Second, the alterations in receptor binding reported here, coupled with essentially normal basal and stimulated levels of dopamine and metabolites following prenatal HAL exposure, may suggest an effect which is postsynaptic to the dopaminergic axons in the neostriatum. The probability that many of the caudate D2 sites are located on acetylcholine (ACh) interneurons (4), coupled with reported alterations of muscarinic cholinergic binding in caudate of PND 30 rat pups exposed prenatally to HAL (10) points to the ACh interneuron as a possible locus of prenatal HAL effects. Future research in this area may, therefore, profitably focus on possible HAL effects upon the number and functional status of the cholinergic components of the nigrostriatal DA system.

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