Pharmacology Biochemistry & Behavior, Vol. 33, pp. 1–6. © Maxwell Pergamon Macmillan plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

Effects of Prenatal Nicotine Exposure on Rat Striatal Dopaminergic and Nicotinic Systems

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Received 7 October 1988

FUNG, Y. K. AND Y.-S. LAU. *Effects of prenatal nicotine exposure on rat striatal dopaminergic and nicotinic systems.* PHARMACOL BIOCHEM BEHAV **33**(1) 1–6, 1989.—Previously we observed that chronic nicotine-treated adult rats developed locomotor hyperactivity which was mediated by changes in nicotinic and dopaminergic receptors in the striatum. In this study, we further examined if such changes would also occur in pups that were prenatally exposed to nicotine. Fourteen-day-old offspring from dams which were implanted with osmotic minipumps containing nicotine (1.5 mg/kg/day) throughout the entire gestational period were employed in this investigation. Prenatal nicotine treatment lowered the number of male pups born and reduced the postnatal gain in body weight and length of both male and female offspring. Prenatal exposure to nicotine did not alter the motor coordination of the pups. A decrease in the number of striatal dopaminergic receptor binding sites (B_{max}) was detected in the male pups, however an increase in the ligand affinity to the receptors ($1/K_D$) had been simultaneously detected. No change in the characteristics of nicotinic receptor binding sites and the levels of dopamine and its metabolite, 3,4-dihydroxyphenylacetic acid was found in the striatal region. The present study indicates that prenatal exposure to nicotine may cause changes in growth and development of the animals. However, in comparison to chronic nicotine-treated adult rats, prenatal exposure to nicotine had only modified the dopaminergic receptor system in the striatal region of male offspring.

Dopamine Nicotine Striatum Receptor

SMOKING or chewing tobacco continually delivers nicotine to the central nervous system. Nicotine administration has elicited locomotor hyperactivity in humans and laboratory animals (3, 5, 11, 16). Several studies suggest a strong correlation between exposure to nicotine in utero via maternal smoking and the increased incidence of attention deficit disorder (ADD) which includes involuntary hyperactivity, restlessness, short attention span and a variety of cognitive and perceptual problems (4, 5, 7, 8, 26). Mothers of hyperkinetic children have been reported to smoke an average of 23 cigarettes a day during the pregnancy period (1, 3, 33). Although the specific etiology of ADD remains unclear, nicotine has been implicated in this disorder due to altering the normal development of the dopaminergic (DAergic) neurons in the striatum, an important area involved in motor functions (3, 5, 14, 30). However, the exact mechanisms whereby nicotine may act to alter the striatal DAergic system remain unclear.

A number of animal studies have described the effects of prenatal nicotine exposure on behavioral development in offspring (2, 14, 24–27). In most of these studies, high doses of nicotine were administered to pregnant animals either by repeated parenteral injections once or twice daily or through drinking water. These methods of administering nicotine to pregnant animals may expose both the mother and fetuses to sudden spike concentrations of nicotine. It has been suggested that these spikes of nicotine can produce hypoxia in the fetuses due to constriction of maternal vasculature (3, 22, 35). In order to circumvent these problems and to deliver nicotine at a dose that closely resembles the amount of nicotine intake as in the case of cigarette smoking in humans, we have administered nicotine to the pregnant rat by the subcutaneous implantation of an osmotic minipump containing nicotine at a dose of 1.5 mg/kg/day. This dose of nicotine has been reported to produce plasma nicotine levels in rats comparable to the plasma level of nicotine detected in humans who smoke one pack of cigarettes a day (11, 13, 15, 22, 28). Thus, the condition of this study is closely mimicking that of human smoking. The effects of prenatal exposure to nicotine on behavior and striatal neurochemistry in 14-day-old pups were examined.

METHOD

Animals

Sprague-Dawley rats (Sasco, Inc. Omaha, NE) weighing about 230 g were used. They were allowed free access to food (Purina Lab. Chow) and water and maintained on a normal 12-hr light/12-hr dark cycle at 21 ± 1°C.

Mating and Gestational Treatment

Male and female rats were paired for mating and the detection of a vaginal plug was designated as gestation day 1. Females were anesthetized with a mixture of halothane and oxygen. An incision was made in the skin posterior to the shoulder and an osmotic minipump Model 2ML4 (Alza Corp., Palo Alto, CA) containing either sterile physiological saline or nicotine (1.5 mg/kg/day) was implanted subcutaneously. The incision was closed with a wound clip and covered with a mixture of benzocaine and Betadine ointment to alleviate discomfort and to prevent infection. The model 2ML4 osmotic minipump contains 2 ml of solution and has a pumping life of 28 days. The dose of nicotine was calculated in terms of free base, using nicotine tartrate (Sigma Chemical Co., St. Louis, MO) dissolved in sterile physiological saline solution. The change in body weight, food and water consumption of all pregnant animals was monitored throughout the entire gestational period. After birth (postnatal day 1), the number, viability, sex ratio, birth weights and body length of all pups in each litter were determined. The saline- and nicotine-exposed pups were cross-fostered to drug-free (surrogate) females who had delivered litters at the same time. This was done to limit the effect of nicotine to the prenatal period.

Determination of Plasma Levels of Nicotine and Cotinine

After delivery, mother rats were killed and trunk blood was collected for the radioimmunoassays of nicotine and cotinine (13,17). Blood samples from 14-day-old male and female pups were also collected and analyzed for the plasma levels of nicotine and cotinine.

Neurobehavioral Testing

Animal behavior such as position reflex, surface righting and negative geotaxis, was used to assess the development of several reflexive parameters, many of which require a certain degree of interlimb coordination and thus determine locomotor performance (34). Behavioral testing of pups was performed on postnatal days 5, 9 and 14. In order to pass the position reflex test, the pup had to assume a normal position within 10 seconds when the hind leg was lowered over the edge of a horizontal table. To pass the surface righting test, the pup had to turn right-side up with all four limbs in a normal standing position within 15 seconds when placed on its back in a supine position on a level surface. To pass the negative geotaxic test, the pup was placed head downward on a textured surface with a 25° slope and was allowed a maximum of 60 seconds to make a 180° turn.

L-[^{3}H]Nicotine Binding Assay

The tissue preparation and L-[^{3}H]nicotine binding assay using rat striatal membranes were carried out according to the methods described by Marks *et al.* (19). Rat striata were dissected and homogenized in 0.5 ml of a buffer containing HEPES (20 mM), NaCl (118 mM), KCl (4.8 mM), $CaCl_2$ (2.5 mM), $MgSO_4$ (1.2 mM) and NaOH for adjusting the pH to 7.5. The homogenate was incubated for 5 min at 37°C and was then centrifuged at 17,500 × g for 30 minutes. The pellet was suspended and lysed in 20 volumes of ice-cold glass distilled water at 4°C for 1 hour, followed by incubating at 37°C for 5 minutes. The sample was centrifuged as above. The pellet was resuspended and washed twice in the original volume of the homogenizing buffer. Membranes were used for the L-[^{3}H]nicotine binding assay.

The L-[^{3}H]nicotine binding assay (in final volume of 0.25 ml) contained Tris HCl (0.2 M), pH 7.5, a single concentration of L-[^{3}H]nicotine (9 nM) and 0.1 ml of striatal membranes (0.4 to 0.7 mg protein). The assay tubes were incubated at 4°C with agitation for 90 minutes. The incubation was terminated by pipetting 3 ml of the homogenizing buffer into the tube and this mixture was poured immediately (within 3 sec) onto a GF/C glass microfiber filter disc (Whatman, Inc., Clifton, NJ) that had been presoaked in 0.5% polyethyleneimine in the homogenizing buffer. The filtration was carried out at 4°C under constant vacuum (5 inches of Hg). Each filter disc was washed three times with 3 ml buffer, dried and counted for radioactivity in a liquid scintillation counter. The nonspecific ligand binding activity was determined by adding cold L-nicotine (1 μM) in each experiment and this value was subtracted from the total binding activity in order to obtain the number of specific L-[^{3}H]nicotine binding sites.

[^{3}H]Spiperone Binding Assay

Striata from the nicotine-exposed or saline-exposed pups were homogenized in 4 ml of 50 mM Tris-HCl buffer (pH 7.4, 4°C) containing 0.1% ascorbic acid, 0.01 mM pargyline, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$. The homogenate was centrifuged at 27,000 × g for 20 min at 4°C. The pellet was additionally washed twice with the same buffer in the same manner. [^{3}H]Spiperone binding assay was performed as previously described (18) using (+)-butaclamol (1 μM) for determining the nonspecific binding sites and mianserin (1 μM) for masking [^{3}H]spiperone binding to 5-hydroxytryptamine receptors. The binding parameters (K_D and B_{max}) of the high affinity [^{3}H]spiperone binding sites were determined by using the nonlinear least-squares regression LIGAND analysis (21).

Determination of Striatal Levels of Dopamine and 3,4-Dihydroxyphenylacetic Acid (DOPAC)

Isolated striata from nicotine-exposed or saline-exposed pups were suspended in 0.2 ml of 0.2 N perchloric acid. The sample was sonicated and centrifuged at 11,000 × g for 5 min at 4°C. The supernatant was filtered through a nylon syringe filter unit (0.45 micron). An aliquot of the filtrate was injected into a high pressure liquid chromatography (Waters, Milford, MA) in a mobile phase consisting of 100 mM sodium acetate, 20 mM citric acid, 100 mg/l sodium octyl sulfate (Eastman Organic Chemicals, Rochester, NY), 50 mg/l EDTA and 4% (v/v) methanol, pH 4.1. The sample was chromatographed by μBondapak C_{18} reverse phase column (3.9 × 150 mm, Waters, Milford, MA) at a constant flow rate of 2 ml/min. Dopamine and DOPAC concentrations in the samples were determined by electrochemical detection at a potential of 0.6 V.

Chemicals

Nicotine tartrate was purchased from Sigma Chemical Company (St. Louis, MO). Both L-[^{3}H]nicotine (60.4 Ci/mmole) and [^{3}H]spiperone (23.2 Ci/mmole) were purchased from New England Nuclear (Boston, MA).

Statistics

Postnatal data are presented as mean ± S.E.M. The pups from each dam were considered to represent a single determination. Analysis of variance (ANOVA) with repeated measures was used

TABLE 1

MATERNAL NICOTINE TREATMENT: EFFECTS ON GESTATION TIME, LITTER SIZE, SEX, WEIGHT AND LENGTH OF OFFSPRING

	Saline	Nicotine
Gestation time (days)	21.4 ± 0.3	21.1 ± 0.3
Litter size		
(No. of pups/litter)	12.8 ± 0.3	9.5 ± 0.5*
No. of Males	6.5 ± 0.4	3.8 ± 0.4*
No. of Females	6.3 ± 0.4	5.7 ± 0.3
Sex Ratio (M/F)	1.0 ± 0.1	0.7 ± 0.1†
Number dead at birth	0	0
Body weight (g)		
Male	6.5 ± 0.2	6.5 ± 0.1
Female	6.1 ± 0.2	6.2 ± 0.2
Body length (cm)		
Male	5.0 ± 0.1	5.0 ± 0.1
Female	5.1 ± 0.1	5.1 ± 0.4

Pregnant animals were implanted with osmotic minipumps containing either physiological saline or nicotine (1.5 mg/kg/day) throughout the entire gestational period. Measurements were taken at birth. Results are mean ± S.E.M., n = 20 litters.

*Significantly different from saline-exposed pups, $p<0.05$ (Student's t-test).

†Significantly different from saline-exposed pups, $p<0.05$ (Mann-Whitney U-test).

TABLE 2

MATERNAL NICOTINE TREATMENT: EFFECTS ON BODY WEIGHT AND LENGTH OF OFFSPRING (POSTNATAL DAYS 5–14)

Offspring (Days after birth)	Body Wt. (g)		Body Length (cm)	
	Saline-Exposed	Nicotine-Exposed	Saline-Exposed	Nicotine-Exposed
Male (5 days)	10.6 ± 0.4	10.0 ± 0.4	6.7 ± 0.1	6.2 ± 0.1*
Female (5 days)	9.7 ± 0.4	8.7 ± 0.3	6.5 ± 0.1	6.1 ± 0.1*
Male (9 days)	18.3 ± 0.7	14.9 ± 0.5*	7.7 ± 0.1	7.3 ± 0.1*
Female (9 days)	18.0 ± 0.4	13.8 ± 0.4*	7.5 ± 0.1	7.1 ± 0.1*
Male (14 days)	26.0 ± 0.7	22.1 ± 0.6*	9.1 ± 0.1	8.5 ± 0.1*
Female (14 days)	25.3 ± 0.5	17.8 ± 1.0*	8.9 ± 0.1	8.2 ± 0.1*

Pregnant animals were implanted with osmotic minipumps containing either physiological saline or nicotine (1.5 mg/kg/day) throughout the entire gestational period. At birth, all pups were cross-fostered to drug-free surrogate mothers. Body weight and body length of all pups were determined at postnatal days 5, 9 and 14. Results are mean ± S.E.M. of 20 litters.

*Significantly different from saline-exposed controls ($p<0.05$).

to determine statistical significance among age, sex and different drug treatments followed by Mann-Whitney U-test, Dunnett's t-test or two-tail Student's t-test when appropriate. Chi-square test was used to analyze the results on motor development. A p level less than 0.05 was considered to be statistically significant.

RESULTS

Plasma Levels of Nicotine and Cotinine

Direct analyses of nicotine and its major metabolite cotinine in maternal blood confirmed effective delivery of nicotine via this route of drug administration. In addition, determination of plasma levels of nicotine and its major metabolite cotinine showed consistency from animal to animal using osmotic minipumps. Gestational treatment of pregnant rats with nicotine (1.5 mg/kg/day) produced a plasma level of nicotine (20.0 ± 2.0 ng/ml) which is comparable to the plasma level reported in humans smoking a pack of cigarettes a day. However, neither nicotine nor cotinine could be detected in the 14-day-old male and female pups (data not shown).

Effect of Nicotine Exposure on Body Development of the Offspring

Maternal nicotine exposure had no significant effect on gestation time (Table 1). However, the number of male pups born from nicotine-treated mothers was significantly reduced (Table 1). With respect to body weight reduction due to prenatal exposure to nicotine, Wilk's criterion F statistics was, $F(3,36) = 7.8$, for male pups and $F(3,36) = 14.9$, for female pups (Table 2). In regards to change in body length, Wilk's criterion F statistics was, $F(3,36) = 5.5$, for male pups and, $F(3,36) = 56$, for female pups (Table 2). All F values are statistically significant at $p<0.05$. Thus, our results confirmed a significant negative influence of maternal nicotine exposure on the growth of offspring as evidenced by a reduction of body weight and length of pups in a time-dependent manner.

Effect of Nicotine Exposure on Locomotor Performance

Both the saline- and nicotine-exposed pups demonstrated

TABLE 3

MATERNAL NICOTINE TREATMENT: EFFECTS ON MOTOR DEVELOPMENT OF OFFSPRING

	Saline (No. pass/No. tested)		Nicotine (No. pass/No. tested)	
	Male	Female	Male	Female
Postnatal day 5				
Righting reflex	20/20	20/20	19/20	18/20
Position reflex	13/20	17/20	11/20	14/20
Negative geotaxis	14/20	13/20	11/20	11/20
Postnatal day 9				
Righting reflex	20/20	20/20	20/20	20/20
Position reflex	14/20	16/20	15/20	16/20
Negative geotaxis	20/20	20/20	20/20	20/20
Postnatal day 14				
Righting reflex	20/20	20/20	20/20	20/20
Position reflex	20/20	20/20	20/20	20/20
Negative geotaxis	20/20	20/20	20/20	20/20

Pregnant animals were implanted with osmotic minipumps containing either physiological saline or nicotine (1.5 mg/kg/day) during the entire gestational period. At birth, all pups were cross-fostered to drug-free surrogate mothers. Motor development of saline- and nicotine-exposed pups was determined on postnatal days 5, 9 and 14.

The results between the saline- and nicotine-exposed pups are not statistically significant (Chi-Square test, $p>0.05$).

TABLE 4

EFFECTS OF MATERNAL NICOTINE EXPOSURE ON STRIATAL NICOTINIC AND DOPAMINERGIC BINDING SITES IN OFFSPRING

Offspring	L-[^{3}H]Nicotine Bound (fmole/mg)	[^{3}H]Spiperone Binding B_{max} (fmole/mg)	[^{3}H]Spiperone Binding K_D (nM)
Saline-exposed male	19.7 ± 3.0	54.1 ± 6.0	0.11 ± 0.01
Nicotine-exposed male	18.7 ± 3.0	28.8 ± 5.4*	0.04 ± 0.01*
Saline-exposed female	17.4 ± 3.0	48.5 ± 6.1	0.10 ± 0.01
Nicotine-exposed female	19.3 ± 2.8	52.6 ± 5.8	0.14 ± 0.03

Pregnant animals were implanted with osmotic minipumps containing either physiological saline or nicotine (1.5 mg/kg/day) for the entire gestational period. At birth, all pups were cross-fostered to drug-free surrogate mothers. Pups were killed on postnatal day 14 and their striata were used for nicotinic and dopaminergic receptor binding assays. Values are mean ± S.E.M. of 5–6 determinations.

*Significantly different from saline-exposed controls ($p<0.05$ Student's t-test).

TABLE 5

EFFECT OF MATERNAL NICOTINE EXPOSURE ON THE STRIATAL LEVELS OF DOPAMINE AND 3,4-DIHYDROXYPHENYLACETIC ACID OF OFFSPRING

Sex	Pretreatment	DA (μg/g)	DOPAC (μg/g)
Male	Saline	4.67 ± 0.30	0.34 ± 0.02
Male	Nicotine	5.10 ± 0.49	0.45 ± 0.04
Female	Saline	4.39 ± 0.27	0.36 ± 0.05
Female	Nicotine	4.38 ± 0.26	0.36 ± 0.02

Pregnant animals were implanted subcutaneously with osmotic minipumps containing either physiological saline or nicotine (1.5 mg/kg/day) for the entire gestational period. At birth, all pups were cross-fostered to drug-free surrogate mothers. Pups were killed on postnatal day 14 and their striata were used for the determination of DA and DOPAC concentrations. Values are mean ± S.E.M. of 5 pups.

The results between the saline- and nicotine-exposed groups are not statistically different ($p>0.05$, Student's t-test).

similar performance on locomotor development as assessed by surface righting, position reflex and negative geotaxic tests (Table 3).

Effect of Nicotine Exposure on Striatal L-[^{3}H]Nicotine Binding Sites

L-[^{3}H]Nicotine was used to quantitate the nicotinic receptor binding sites in the pup striatum. As discussed previously (9), due to tissue limitation and low density of nicotinic binding sites in the striatum, only a single dose of L-[^{3}H]nicotine (9 nM) was used in our studies to label the receptors in the pups. We did not detect a significant difference in the binding of L-[^{3}H]nicotine to the receptor sites in striatal membranes between the saline- and nicotine-exposed pups (Table 4).

Effect of Nicotine Exposure on Striatal [^{3}H]Spiperone Binding Sites in Pups

[^{3}H]Spiperone was used in this study to quantitate the dopamine receptor binding sites in the striatum. A dose-dependent [^{3}H]spiperone (0.1–5 nM) binding curve was determined in each of the control and nicotine-exposed pups and their respective K_D and B_{max} were compared. A reduction in the number of DAergic receptor binding sites as well as an increase in the affinity of these receptors were detected in the male nicotine-exposed pups (Table 4).

Nicotine Exposure on Striatal DA and DOPAC Levels

We further examined the effect of nicotine exposure on striatal DA and DOPAC concentrations in the 14-day-old pups. Our results showed that prenatal exposure to nicotine did not alter the striatal contents of DA and its major metabolite DOPAC (Table 5).

Nicotine Treatment on Body Weight, Food and Water Consumption in Pregnant Rats

The effects of continuous nicotine administration via osmotic minipumps on body weight, food and water consumption in pregnant rats were determined throughout this study. We found an increase in body weight in both groups of rats, however, the gain in body weight in saline- and nicotine-treated rats was at the same rate. In addition, we did not find a difference in saline- and nicotine-treated animals in terms of their daily food and water consumption (data not shown).

DISCUSSION

Other studies have shown that high doses of nicotine can affect body weight, food and water consumption in laboratory animals (12,22). In our study, nicotine which was administered to pregnant rats at a dose of 1.5 mg/kg/day via osmotic minipumps, had no effect on daily gain in maternal body weight, food and water consumption. Implantation of osmotic minipumps did not cause any overt sign of infection or tissue necrosis. Both nicotine and cotinine were detected in the plasma of nicotine-treated mothers after delivery, but not in the plasma of 14-day-old pups which were nursed by drug-free surrogate mothers after birth. Furthermore, the plasma level of nicotine found in the pregnant rats was comparable to the plasma nicotine levels found in an individual who smokes one pack of cigarettes a day (10, 13, 22). Therefore, the present study provides information on the effects of nicotine under conditions in which nicotine intake more closely resembles cigarette smoking in humans. In our previous studies, we observed that the 14-day-old nicotine-exposed pups were spontaneously hyperactive (10). Thus, in the present studies, all biochemical determinations were conducted on 14-day-old offspring.

Maternal nicotine treatment had no significant effect on gestation time. However, the number of male offspring born to nicotine-treated mothers was significantly reduced when compared to saline-treated controls. This finding is in agreement with other animal studies which reported an appreciable reduction in male offspring of nicotine-treated mothers (2, 23, 24, 26). In the offspring from nicotine-treated mothers, a decrease in growth rate was observed particularly after postnatal day 9. This observation is consistent with recent studies which showed that gestational nicotine exposure had a profound effect on cell maturation (29,32). Both the saline- and nicotine-exposed pups showed similar ability in performing the surface righting, position reflex and negative neotaxic tests. This suggests that prenatal exposure to nicotine did not have an effect on their motor performances.

Several studies have shown that continuous administration of

nicotine for 7 to 10 days resulted in an up-regulation in the number of striatal nicotinic receptors in adult animals (19,20). In our 5-day chronic nicotine study, we observed an increase in the nicotine-stimulated locomotor response which was associated with an increase in the number of L-[^{3}H]nicotine binding sites and an elevated DA level in the striatum (9). Furthermore, in animals which were pretreated with nicotine (1.5 mg/kg/day) for 14 days, the nicotine-induced locomotor response was still potentiated and this was correlated with an increase in the number of striatal [^{3}H]spiperone binding sites (9).

This study was designed to examine if maternal exposure to nicotine would alter the striatal nicotinic and dopaminergic receptor systems in the offspring. It is of particular interest to compare the prenatal effects of nicotine on offspring to its direct effects on adult animals. We did not find significant change in the number of nicotinic receptor binding sites as well as the levels of DA in the striatum of 14-day nicotine-exposed pups. Other investigators have also failed to detect changes in adrenal medullary nicotinic receptors in the offspring from nicotine-treated mothers (27). However, increases of nicotinic receptor binding sites have been reported in various brain regions of nicotine-exposed offspring including midbrain + brainstem, cerebral cortex and cerebellum (31). In contrast, we did not detect such a change in the striatum of nicotine-exposed pups at postnatal day 14 though these pups were behaviorally hyperactive (10). Thus, there may be a regional difference in the effect of nicotine. A decrease in the number (B_{max}) and an increase in the affinity ($1/K_D$) of striatal DAergic receptor binding sites were found only in the nicotine-exposed male pups. The increase of DA receptor affinity may functionally correct for the loss of the receptor sites due to nicotine exposure.

In conclusion, the present study demonstrated that prenatal exposure to nicotine induced growth deficit in the offspring. Although nicotine did not modify the characteristics of nicotinic receptor binding sites in the striatum, a change in the number and affinity of DAergic receptor binding sites was observed in male offspring. Furthermore, the similarities between the effects of maternal tobacco smoking on the offspring and that of nicotine administration via osmotic minipumps in rats may suggest that the latter is a useful animal model for smoking in humans.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the technical assistance of J. Reed, A. Schulte and K. Trobough. In addition, we wish to thank Ms. Caryn Axelrad (American Health Foundation, New York) for the analysis of nicotine and cotinine in plasma samples and Dr. Norman Uretsky for his suggestions in the preparation of this manuscript. This study was supported by a grant (No. 88-13) from the Department of Health, State of Nebraska.

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