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Prenatal Nicotine Exposure: Effects on Locomotor Activity and Central [^{125}I]α-BT Binding in Rats

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TIZABI, Y., L. T. RUSSELL, S. M. NESPOR, D. C. PERRY AND N. E. GRUNBERG. *Prenatal nicotine exposure: Effects on locomotor activity and central [^{125}I]α-BT binding in rats*. PHARMACOL BIOCHEM BEHAV **66**(3) 495–500, 2000.—Maternal smoking during pregnancy or in utero exposure of the fetus to nicotine may result in learning difficulties and hyperactivity in the child. To elucidate possible involvement of the α_7 nicotinic receptor subtype in these behavioral impairments, pregnant dams were treated with nicotine (9 mg/kg/day) via osmotic minipumps throughout gestation. Male offspring were weaned at postnatal day 18, and were tested for locomotor activity at postnatal days 20–24. Pups were sacrificed on postnatal day 36–38 and 18 discrete brain areas were analyzed for [^{125}I]alpha-bungarotoxin (α-BT) binding by quantitative autoradiography. Prenatal nicotine caused an elevation in locomotor activity (vertical movements) in offspring. [^{125}I]α-BT binding was significantly reduced in the hippocampal CA1 region (29%), dentate gyrus (22%), and medial geniculate nucleus (29%). These findings suggest that some of the behavioral abnormalities induced by prenatal nicotine exposure may be due to a reduction of α_7 nicotinic receptors in discrete brain regions. © 2000 Elsevier Science Inc.

Prenatal Nicotine Nicotinic receptors Autoradiography Locomotor activity Hippocampus Rat

BEHAVIORAL and cognitive impairments in children as a result of prenatal exposure to nicotine through maternal smoking are well supported by epidemiological studies (5,10–12,18,27,30,49). Similarly, animal models of in utero exposure to nicotine have demonstrated behavioral, neurochemical, and cognitive abnormalities in offspring (13,19,20,24,26,31, 34,37,41,44,46,51). These effects of nicotine are most likely mediated by nicotinic receptors that are present in fetal brain by midgestation, and are believed to play a critical role in neuronal differentiation and development (3,6,16,44,52).

Various nicotinic receptor subtypes with distinct developmental, physiological, and pharmacological properties have been identified (9,16,21,43,57). The two prominent and most extensively studied central nicotinic receptors include the alpha4-beta2 and the homomeric alpha7 subtypes. Recently, Tizabi et al. (51) reported that hyperactive male, but not female, offspring of dams treated with nicotine (6 mg/kg/day throughout gestation) had an increased [^{3}H]cytisine binding in the cortex. [^{3}H]Cytisine is a ligand specific for α4β2 receptor subtype. Because of postulated roles of alpha7 nicotinic receptor in neuronal growth and development (35,36,43,57), this study was undertaken to evaluate the effects of prenatal nicotine exposure on locomotor activity and the density of alpha7 receptors in discrete brain regions of the male offspring.

METHOD

Animals and Treatments

Timed-pregnant Sprague–Dawley rats weighing 190–230 g were purchased from Charles River Laboratories (Kingston, NY). The animals were maintained in an environmentally controlled room with a 12 L:12 D cycle (lights on at 1900 h), a

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temperature range of 22–24°C, and relative humidity of approximately 50%. Subjects were housed individually in 35.6 × 15.2 × 20.3-cm cages with absorbent Pine-Dri, wood-chip bedding, and had continuous access to standard laboratory chow (Agway Prolab 3000) and water. The experimental protocol was approved by the Institutional Review Committee for the use of Animal Subjects. The procedures applied were in compliance with the National Institutes of Health Guides for Care and Use of Laboratory Animals (Pub No. 85-23, revised 1985).

On the fourth day of gestation, 12 pregnant dams were randomly divided into two groups (six/group) to receive either nicotine dihydrochloride (9 mg/kg/day nicotine base dissolved in physiological saline) or saline via osmotic minipumps (model 2002, Alza Corp., Palo Alto, CA) implanted subcutaneously in the intra scapular space. Methoxyflurane (Metophane), administered by inhalation, was used to anesthetize the dams during the implantation surgery. The minipumps, with a flow rate of 0.48 μl/h and a fill volume of 238 μl, had a total delivering capacity of approximately 23 days.

Previously, we used 3 and 6 mg/kg/day of nicotine in studying the prenatal effects of nicotine on similar behavioral paradigms (37,51). An overall statistically significant effect on horizontal locomotor activity of the pups was not detected with such doses of nicotine. In this study, the higher dose of 9 mg/kg/day was used to determine whether an overall behavioral effect could be detected and whether the behavioral changes could be correlated with the neurochemical effects.

Plasma levels of nicotine and cotinine (the major metabolite of nicotine) in pregnant rats administered nicotine (3 and 6 mg/kg/day) was reported earlier (37). Plasma nicotine concentrations of dams treated with 6 mg/kg/day nicotine were 102–107 ng/ml (37). Plasma nicotine concentrations of rats treated with 12 mg/kg/day nicotine were recently reported to be approximately 250 ng/ml (54). Hence, the plasma nicotine concentrations of dams treated with 9 mg/kg/day are expected to range 150–200 ng/ml.

All pups were born by day 23 of gestation, and were weaned on day 18 postnatally. Male littermates were housed in groups of three to four pups per cage. A total of 46 male pups (24 from four dams treated with saline, and 22 from four dams treated with nicotine) were used in this study. Litters were selected from matched nicotine- and saline-treated dams such that the same or very close number of male offspring per dam could be obtained.

Locomotor Activity Testing

Locomotor activity was measured using an Omnitech Electronics Digiscan infrared photocell system (Test box model RXYZCM, 16 TAO, Omnitech Electronics, Columbus, OH) where animals were placed singly in a 20 × 20 × 30-cm clear Plexiglas arena. The tests were conducted on days 20, 21, 23, and 24 postnatally during the dark portion of the rats' activity cycle (0800–1400 h). Spontaneous locomotor activity, determined by the total horizontal distance traveled, as well as number of vertical movements, were automatically gathered and transmitted to a personal computer via an Omnitech Model DCM-8-BBU analyzer. Animals were monitored continuously for 60 min on each testing day with data recorded as cumulative activity over 5-min time periods.

Brain Collection

All pups were sacrificed at age 36–38 days, an age when the nicotinic binding capacities of rat brain membranes have achieved their adult levels and are no longer subject to changes with age (42). The order of sacrifice was alternated between the two groups. Following decapitation, brains were rapidly removed and frozen in powdered dry ice and kept at −80°C until receptor analysis.

Nicotinic Receptor Measurement

Quantitative autoradiography as described in detail by Clarke et al. (7), Sorenson and Chiappinelli (47), and Perry et al. (33), was used to measure [^{125}I]alpha-bungarotoxin (α-BT) binding in 18 discrete brain regions. Briefly, sagittal sections (16 μm thick), were cut in a cryostat, thaw mounted on gelatin-coated slides, dried under partial vacuum at 4°C for 12 h, and stored at −80°C for at least 24 h. Frozen slide-mounted brain sections were preincubated at room temperature for 30 min in 50 mM Tris-HCl buffer, pH 7.4, containing 1 mg/ml of bovine serum albumin. This was followed by 2-h incubation at room temperature in the same buffer containing approximately 0.5 nM [^{125}I]α-bungarotoxin. Nonspecific binding was assessed in presence of 100 μM (−)-nicotine. Sections were washed three times (10 min each) in 500 ml of cold (4°C) buffer, dried, and stored in cassettes for 7 days. Autoradiographs were analyzed by Loats Inquiry image analysis software. It has been established that [^{125}I]alpha-bungarotoxin binds specifically to the homomeric alpha-7 nicotinic receptor subtype (4).

Data Analysis

Maternal and offspring weights as well as locomotor data were analyzed by repeated-measures ANOVA, with time and treatments as variables. One-way ANOVA was used to examine the effects of prenatal nicotine exposure on nicotinic receptor binding. When a significant main (treatment) or interaction effect (treatment × time or treatment × region) was observed, the Newman–Keuls post hoc test was applied to identify the specific time (day) or region affected. All analysis were two tailed, and used an alpha of 0.05 or less to determine significance.

RESULTS

Effects on Maternal Weight

Dams receiving nicotine had significantly lower weight gain throughout gestation compared to control. This difference was most apparent at gestation day 18 when the average weight gain of nicotine treated dams was approximately 82% of control (93 g gain for nicotine and 113 g for control. The actual weights at gestation day 18 were significantly different between the two groups: controls = 326 ± 10, nicotine treated = 298 ± 8 (mean ± SEM), $F(1, 10) = 4.99$, $p < 0.05$.

Delivery Outcome and Offspring Weight

All dams delivered successfully. Although nicotine-treated dams had fewer number of total pups (60 pups) compared to control (71 pups), the mean number of pups per dam was not statistically different between the two groups (control = 11.83 ± 0.48, nicotine exposed = 10.3 ± 0.95, mean ± SEM, $p = 0.19$). However, the average birth weight of nicotine-exposed pups was approximately 15% lower than the control (actual weights: nicotine exposed = 7.0 ± 0.35, control = 8.2 ± 0.32 g (mean ± SEM), $F(1, 1290 = 8.25$, $p < 0.01$. The difference in weight was still apparent in 20-day-old offspring (38.5 vs 43.9 g average weight in nicotine-exposed and control, respectively).

However, at postnatal day 35, no apparent difference in weight was observed between the two groups (139 vs. 140 g average weight in nicotine-exposed and control, respectively). Thus, it appears that the initial reduction in birth weight is gradually overcome during the postnatal developmental period. This might be due to an increase in food and water intake in nicotine-exposed pups.

Effects on Locomotor Activity

Figure 1A presents the horizontal activity (total distance traveled) for nicotine-preexposed pups and their control. Pups exposed to nicotine in utero had a slight increase in daily horizontal locomotion as well as mean cumulative activity (approximately 18%) that did not attain statistical significance.

Figure 1B presents the vertical activity (number of vertical movements) for nicotine-preexposed pups and their control. Pups exposed to nicotine in utero had significant increases in daily (3 of the 4 test days) as well as mean cumulative activity (approximately 26%, $p < 0.05$).

Locomotor activities (horizontal and vertical) were highest during the first test day in both treated and control animals. This was probably due to the novelty of the environment during the first test day, to which adaptation occurred in subsequent test days.

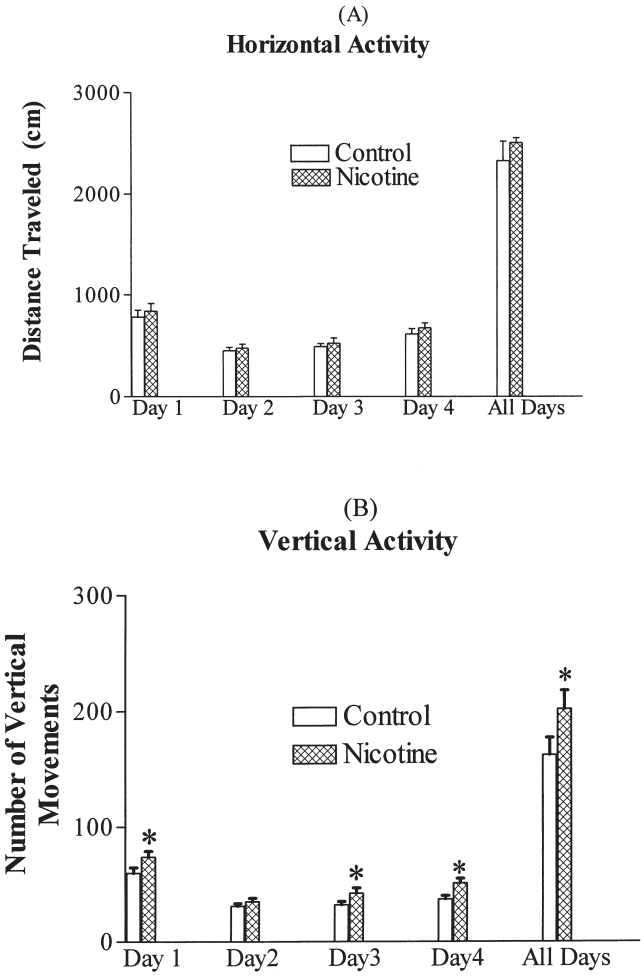

FIG. 1. This presents the effect of prenatal nicotine administration (9 mg/kg/day during gestation) on horizontal (A) and vertical (B) activity of male offspring. Pups were weaned on postnatal day 18, and were tested for locomotor activity on days 20 (day 1), 21 (day 2), 23(day 3), and 24 (day 4). Cumulative scores for all test days is presented in the last column. Values are Mean ± SEM, n = 22–24/group. *$p < 0.05$ compared to control.

Effects on [^{125}I]α-BT Binding

Table 1 presents the effects of prenatal nicotine exposure on [^{125}I]α-bungarotoxin binding in discrete brain regions. Pups exposed to nicotine prenatally had a decrease in [^{125}I]α - BT binding in the 18 regions examined. A significant drug effect, $F(1, 765) = 4.53$, $p < 0.05$, as well as interaction (treatment × region), $F(1, 16) = 9.6$, $p < 0.01$ was observed. Post hoc analysis revealed significant decreases in hippocampal CA1 (29%, $p < 0.05$), dentate gyrus (20%, $p < 0.05$), and medial geniculate nucleus (29%, $p < 0.05$).

It should be noted that because only a single concentration of the ligand was used to measure the binding, the possibility that changes may be occurring in the binding affinity of the receptors can not be ruled out.

DISCUSSION

The results of the current study indicate that prenatal exposure to nicotine in rats can result in behavioral changes, manifested in increased vertical movements and neurochemical changes, manifested in nicotinic receptor alterations in discrete brain regions of young offspring. These findings are in agreement with numerous epidemiological and preclinical studies demonstrating detrimental effects of prenatal nicotine exposure. In humans, fetal exposure to nicotine through cigarette smoking during pregnancy can result in learning difficul-

TABLE 1

DENSITY OF [^{125}I] ALPHA-BUNGAROTOXIN BINDING (dpm/mg TISSUE) IN 18 DISCRETE BRAIN REGIONS OF 36–38-DAY-OLD MALE RATS THAT WERE EXPOSED TO NICOTINE (9 mg/kg/day) OR SALINE (CONTROL) THROUGHOUT GESTATION

Region	Control	Nicotine
Caudate putamen	339 ± 10	343 ± 10
Cerebellum (vermis)	366 ± 10	331 ± 15
Dentate gyrus	1813 ± 119	1418 ± 84*
Hippocampus (CA1)	1241 ± 51	884 ± 65*
Hippocampus (CA4)	3131 ± 200	2808 ± 190
Inferior colliculus	2062 ± 168	1920 ± 184
Medial geniculate nucleus	2200 ± 206	1570 ± 65*
Nucleus accumbens	449 ± 12	397 ± 12
Parietal association cortex	536 ± 17	487 ± 15
Primary motor cortex	519 ± 13	497 ± 14
Primary visual cortex	560 ± 17	507 ± 14
Retrosplenial agranular cortex	541 ± 16	488 ± 15
Secondary motor cortex	589 ± 15	539 ± 15
Secondary visual cortex	480 ± 15	457 ± 15
Somatosensory cortex	622 ± 16	565 ± 16
Substania nigra	1748 ± 131	1612 ± 136
Superior colliculus superficial gray layer	2054 ± 161	1696 ± 145
Ventral tegmental area	1610 ± 121	1440 ± 156

Values are mean ± SEM.
n = 18–24.
*$p < 0.05$ compared to control.

ties and hyperactivity during childhood (5,10–12,18,27,30,49). Animal studies have confirmed teratogenic effects of in utero nicotine exposure. Specifically, it has been demonstrated that neuronal replication and differentiation may be compromised, leading to altered synaptic activities in a variety of neurotransmitter systems (39,44).

Nicotine freely crosses the placenta (40), and has been found in the amniotic fluid and umbilical cord of neonates (17,23). Intrauterine exposure to nicotine results in decreases in birth weight, increased incidence of spontaneous abortions, and increased perinatal mortality (8,11,14,22,25,27,28,30). Our findings of reduced offspring numbers and lower birth weights in nicotine exposed pups is compatible with these observations in humans.

Actions of nicotine are mediated through nicotinic receptors that are present during gestation (3,6,15,16,44,52). Developmentally, nicotinic binding sites in rat brain increase by approximately threefold from birth to adulthood, with near maximal binding occurring at 4 weeks (42). However, considerable regional variability in developmental profile in human, rat, and mouse brain is noted (16,43,45,52,57). Moreover, it appears that different nicotinic receptor subtypes may have different developmental profiles (26,43,44,52,57). In general, however, adult concentration levels of the various receptor subtypes in discrete brain regions are already achieved by day 20–30 postnatally (26,29,31,42,45,52). Hence, the observed effects on nicotinic receptors in this study reflect a developmental impact of nicotine on nicotinic receptors.

Intrauterine exposure to nicotine results in upregulation of nicotinic receptors in fetal as well as in postnatal brains (15,45,51,52). This upregulation of receptors may actually represent receptor desensitization and functional downregulation of nicotinic receptors (32,53,55,56). It was demonstrated recently that administration of nicotine (6 mg/kg/day) to pregnant rats results in an increased cortical [^{3}H]cytisine binding in hyperactive male offspring (51). [^{3}H]Cytisine is a ligand specific for the $\alpha4\beta2$ receptor subtype. Although in the same study a higher percentage of hyperactive offspring were obtained from dams treated with nicotine, a statistically significant elevation in mean horizontal activity scores was not obtained in male offspring. Also, in the current study, the increase in total horizontal activity scores in male offspring exposed to 9 mg/kg/day during gestation did not achieve statistical significance (Fig. 1A). However, a significant elevation in vertical movements was observed in these offspring (Fig. 1B). In addition, prenatal nicotine exposure resulted in a reduction in [^{125}I]α-BT binding in several discrete brain regions of male offspring. Although the results of the present experiment preclude assignment of causal relationship between the observed alpha7 nicotinic receptor reduction and hyperactivity in offspring, our findings extend the previously observed neurochemical effects of prenatal nicotine exposure to include changes in the alpha7 nicotinic receptor subtype.

[^{125}I]Alpha-bungarotoxin specifically labels the $\alpha7$ nicotinic receptor subtype (4). These receptors are predominantly presynaptic, and may regulate the release of a variety of neurotransmitters (38,55). In addition, $\alpha7$ nicotinic receptors are believed to have a prominent role in synaptogenesis and neuronal development (36,38,43,57). This receptor subtype is most abundant in the hippocampus, and has been implicated in sensory gating processes (2,48). Abnormal sensory gating may reflect attentional deficit and/or cognitive impairments (1,2,48,50). Our finding of reduction in $\alpha7$ nicotinic receptors in hippocampus and medial geniculate nucleus, areas implicated in sensory processing, and/or cognitive functions, suggests involvement of this receptor in behavioral and/or cognitive abnormalities induced by prenatal nicotine exposure. Clearly, further experiments are required to ascertain this hypothesis. However, the observed reduction in $\alpha7$ nicotinic receptors in this study is congruent with the notion of neuronal or synaptic losses due to prenatal nicotine administration (39,44). Whether neuronal loss is mediated by nicotinic action on fetal $\alpha7$ receptors remains to by investigated.

In summary, prenatal nicotine exposure resulted in an elevation in vertical movements and a reduction in [^{125}I]α-BT binding in the hippocampal CA1 region (29%), dentate gyrus (22%), and medial geniculate nucleus (29%) of male offspring, suggesting that some of the behavioral or cognitive abnormalities induced by prenatal nicotine exposure may be due to a reduction of $\alpha7$ nicotinic receptors in discrete brain regions.

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