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Postnatal effects of maternal nicotine exposure on the striatal dopaminergic system in rats

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Abstract—The biochemical effects of continuous prenatal exposure to nicotine (1·5 mg kg^{-1} day^{-1}) have been examined in 14 day old male and female rat pups. The ability of striatal tissue slices from the nicotine-exposed pups to synthesize [^{3}H]dopamine from [^{3}H]tyrosine was enhanced. However, the ability of (+)-amphetamine to stimulate formation and release of [^{3}H]dopamine from [^{3}H]tyrosine was not affected. Furthermore, prenatal exposure to nicotine increased the rate of striatal dopamine turnover in the male, but not the female nicotine-exposed pups.

Several animal studies have reported the prenatal effects of nicotine exposure in-utero on behavioral development of offspring (Becker & Martin 1971; Hudson et al 1973; Peters et al 1982; Nasrat et al 1986). In general, nicotine exposure of rats during pregnancy altered the survival rate, growth and development of the offspring (Sershen et al 1982; Fielding 1985). These effects can be attributed to the actions of nicotine, with or without contributing factors associated with hypoxia and ischaemia (Benowitz 1986). In most of these studies, high doses of nicotine were administered to pregnant animals by systemic injection which exposes both mother and foetus to sudden high levels of nicotine. High doses of the drug can produce constriction of the placental vasculature resulting in foetal hypoxia (Werler et al 1985; Benowitz 1986).

To avoid delivering a large bolus dose of nicotine, administration was via an osmotic minipump at a dose of 1·5 mg kg^{-1} day^{-1}. This dose of nicotine was chosen as it has been shown to produce plasma nicotine levels in rats similar to those in humans smoking 20 cigarettes a day (Issac & Rand 1972; Murrin et al 1987; Fung & Lau 1989).

Some behavioural effects of nicotine have been suggested to be mediated via stimulation of the nicotinic receptors in the corpus striatum (Giorguieff-Chesselet et al 1979; Marks et al 1986; Fung & Lau 1989). Activation of presynaptic nicotinic receptors at the dopaminergic (DAergic) nerve terminals in striatal and mesolimbic regions has been demonstrated to stimulate the release of dopamine (DA) (Westfall 1983; Martino-Barrows & Kellar 1987; Fung & Lau 1989). Thus, DA may play a role in mediating some of the central nervous system effects elicited by nicotine.

Previously, we reported that prenatal exposure to nicotine induced locomotor hyperactivity in 14 day old offspring (Fung 1988). We now examine the biochemical changes in the striatum of these nicotine-exposed pups.

Materials and methods

Sprague-Dawley rats of either sex (Sasco, Omaha, NE) were housed in groups of 3 per cage (the sexes separate) in a temperature-(23 ± 1°C) controlled environment with a 12 h light–dark cycle and free access to food (Purina Lab Chow, St. Louis, MO) and water.

Subsequently male and female rats were paired for mating and the detection of a vaginal plug was designated as gestation day 1. Females were anaesthetized 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 2 mL of sterile physiological saline or nicotine (1·5 mg free base kg^{-1} day^{-1}) with enough for 28 days was implanted subcutaneously. Nicotine tartrate (Sigma Chemical Co. St Louis, MO) was dissolved in sterile physiological saline solution. The change in body weight, food and water consumption of all pregnant rats was monitored over the gestational period. After birth (postnatal day 1), the litter size, viability, sex ratio, and the birth weights and body length of 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. Fourteen day old male and female saline- and nicotine-exposed offspring were used.

Determination of striatal [^{3}H]dopamine formation and release from [^{3}H]tyrosine. This study was conducted according to Fung & Uretsky (1980). Pups were killed and their striata were dissected, weighed and sliced in 0·25 mm^2 sections dispersed in ice cold normal medium containing (mM) NaCl 118·4, KCl 4·73, KH_2PO_4 1·2, $MgSO_4 \cdot 7H_2O$ 1·18, $CaCl_2 \cdot 2H_2O$ 1·25, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid 22 with dextrose 2 mg mL^{-1}. The solution was bubbled with 95% O_2 and 5% CO_2 for 30 min and adjusted to pH 7·2 with 1 N NaOH. The slices were then centrifuged at 500 *g* for 5 min, the supernatant being discarded. The slices were then resuspended in cold normal medium such that 0·25 mL aliquots would contain 25 mg of the striatal tissue. (+)-Amphetamine (10^{-6} M) was added; to make the final volume of the suspension 3 mL. The slices were incubated under 95% O_2 and 5% CO_2 in a vibrating water bath at 37°C for 8 min. [^{3}H]Tyrosine was then added to a final concentration of 10 μM. The incubation was continued for 30 min and the reaction stopped by cooling the flasks on ice. Replicate slices kept on ice throughout the experiment served as controls. Tissues were separated from the medium by centrifugation and both fractions were assayed for [^{3}H]DA. [^{3}H]DA was separated from [^{3}H]tyrosine by alumina absorption and ion exchange (Amberlite CG 50) chromatography. The radioactivity present was determined by liquid scintillation counting. Total [^{3}H]DA was obtained by summing the activity from tissue and medium fractions. The release of newly synthesized [^{3}H]DA from the tissue was calculated by dividing the amount of [^{3}H]DA in the medium by the total amount of [^{3}H]DA formed.

Determination of dopamine turnover in pup striatum. The rate of DA turnover was measured as the rate at which DA level declined after i.p. administration of the tyrosine hydroxylase inhibitor. α-methyl-*p*-tyrosine (300 mg kg^{-1}, i.p.) (Costa & Neff 1969). Pups were killed at 1·5 and 3 h after injection. Striata from saline- or nicotine-exposed pups were dissected and suspended in 0·2 mL of 0·2 M 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 μm). An aliquot of the filtrate was injected into a high peformance liquid chromatograph (Waters, Milford, MA) in a mobile phase consisting of 100 mM sodium acetate, 20 mM citric acid, 100 mg L^{-1} sodium octyl sulphate (Eastman Organic Chemicals, Rochester, NY), 50 mg L^{-1} EDTA and 4% (v/v) methanol, pH 4·1. The sample was chromatographed by microBondapak C_{18} reverse phase column (3·9 × 150 mm, Waters, Milford, MA) at a constant flow rate of 2 mL min^{-1}. Striatal dopamine levels were determined by electrochemical detection at a potential of 0·6 V.

Drugs. [^{3}H]tyrosine (55 Ci mmol^{-1}) was purchased from Amersham (Chicago, IL). Nicotine tartrate and (+)-amphetamine sulphate were purchased from Sigma Chemical Company (St. Louis, MO). Alzet osmotic minipumps (Model 2ML4) were purchased from Alza Corporation (Palo Alto, CA).

Statistics. All statistical comparisons were made using analysis of variance followed by Newman-Keuls for comparison between and within groups and the two-tailed Student's *t*-test for independent means. A P level less than 0·05 was considered to be statistically significant.

Results

Effect of maternal nicotine-exposure on striatal [^{3}H]dopamine formation and release from [^{3}H]tyrosine. The effect of prenatal exposure to nicotine on the ability of striatal slices to synthesize and release [^{3}H]DA from [^{3}H]tyrosine was examined. The ability of the striatal tissue to synthesize DA was enhanced from pups prenatally exposed to nicotine, although the release of newly synthesized [^{3}H]DA was not affected (Table 1). Addition of (+)-amphetamine to tissue slices significantly stimulated [^{3}H]DA formation and release. However, the stimulatory effects of (+)-amphetamine on [^{3}H]DA formation and release in the nicotine-exposed pups were not significantly different from the saline-exposed controls.

Table 1. Effect of maternal exposure to nicotine on (+)-amphetamine-stimulated formation and release of [^{3}H]DA from [^{3}H]tyrosine in male and female rat pup striatal slices.

Sex	Pretreatment	Drug added to medium	[^{3}H]DA formed (nmol g^{-1}/30min)	% [^{3}H]DA released
Male	Saline	None	1·3±0·1	32·9±2·0
Female	Saline	None	1·2±0·1	29·5±2·0
Male	Nicotine	None	1·9±0·1*	32·8±3·1
Female	Nicotine	None	1·8±0·1*	32·1±1·1
Male	Saline	(+)-Amph.	3·3±0·1	76·3±1·0
Female	Saline	(+)-Amph.	3·3±3·0	76·5±2·1
Male	Nicotine	(+)-Amph.	3·6±0·3	77·2±3·0
Female	Nicotine	(+)-Amph.	3·5±0·2	79·2±1·1

Striatal slices (25 mg) from saline- or nicotine-exposed male and female pups were incubated in normal medium containing no drug or (+)-amphetamine ((+)-Amph. 10^{-6} M). The slices were preincubated for 8 min. [^{3}H]Tyrosine was added and the reaction was continued for 30 min at which time [^{3}H]DA formation and release was determined. Data are presented as mean±s.e.m. of 6–8 determinations.

* Significantly different from the saline-exposed group ($P<0·05$).

Effect of maternal nicotine exposure on striatal DA turnover. The rate of DA turnover in the nicotine-exposed pups at 1·5 h after α-methyl-*p*-tyrosine injection was not significantly different from the saline-exposed controls. However, in the male nicotine-exposed group, striatal DA content was reduced by 61%, whereas in saline-exposed controls the reduction in DA was only 41% 3 h later. The results were statistically significantly ($P<0·05$) (Table 2).

Table 2. Effect of maternal exposure to nicotine on DA turnover in 14 day old offspring.

		DA contents (ng mg^{-1} tissue) at different time (h) after α-methyl-p-tyrosine inj.		
Sex	Pretreatment	0 h	1·5 h	3 h
Male	saline	4·8±0·3	3·2±0·3	2·8±0·2
Male	nicotine	5·1±0·6	3·7±0·4	2·0±0·2*
Female	saline	4·6±0·4	2·7±0·2	2·0±0·3
Female	nicotine	5·3±0·3	3·4±0·4	2·5±0·3

Pregnant animals were implanted subcutaneously with osmotic mini-pumps containing either physiological saline or nicotine (1·5 mg kg^{-1} day^{-1}) for the gestational period. At birth, all pups were cross-fostered to drug-free surrogate mothers. On postnatal day 14, all pups were injected with α-methyl-*p*-tyrosine (300 mg kg^{-1}, i.p.) and killed at 0, 1·5 and 3 h later. The striata were dissected and analysed for the levels of DA using HPLC. Values are presented as mean±s.e.m. of 4 pups.

* Significantly different from the saline-exposed group ($P<0·05$).

Discussion

High doses of nicotine have been shown to affect the body weight as well as food and water consumption in animals (Grunberg et al 1986; Murrin et al 1987). However, we previously reported that nicotine administered to rats at a constant rate by osmotic minipumps, had no effect on daily gain in body weight, or daily food and water consumption (Fung & Lau 1989). Direct maternal blood analyses for nicotine and its major metabolite cotinine, confirmed effective entry of nicotine via this method of nicotine administration. Although nicotine was detected in the nicotine-treated mother (20±2·0 ng mL^{-1}), neither nicotine nor cotinine could be found in 14 day old male or female pups (data not shown).

Several human studies suggest a correlation between exposure to nicotine in-utero and the incidence of attention deficit disorder (ADD) (Denson et al 1975; Shaywitz et al 1976; Brown et al 1985; Fung 1988). Although the specific aetiology of ADD remains unclear, nicotine has been hypothesized to play a role in this disorder by affecting the normal development of the nigrostriatal dopaminergic system. We found that prenatal exposure to nicotine did not affect the motor development of the nicotine-exposed pups, even though these pups were spontaneously hyperactive (Fung 1988). Furthermore, no change in the characteristics of nicotinic receptor binding sites was found in the striatal regions of either male or female nicotine-exposed offspring (data not shown).

We further examined some of the biochemical effects of prenatal nicotine exposure on striatal DAergic neurons. Our earlier study showed that pretreatment of adult rats with nicotine (1·5 mg kg^{-1} day^{-1}) for 14 days did not alter the stimulatory effects of (+)-amphetamine on [^{3}H]DA synthesis and release in tissue slices from rat striatum (Fung & Lau 1989). Interestingly, the ability of striatal tissues from 14 day old pups to synthesize [^{3}H]DA was enhanced. However, the stimulatory effect of (+)-amphetamine on [^{3}H]DA formation or release by the striatal slices was not altered.

Chronic administration of nicotine to rats has been shown to decrease the rate of DA turnover in adults (Fuxe et al 1986; Fung & Lau 1989). In contrast, we detected an increase in the rate of DA utilization only in the male pups 3 h following α-methyl-*p*-tyrosine administration. Thus, it is possible that prenatal exposure to nicotine has a greater effect on the male than female offspring. To support this, we have found that the number of male offspring (4·2±0·4) born to nicotine-treated mothers was significantly reduced when compared with saline-treated controls (6·5±0·4). This is in agreement with other reports (Peters et al 1982; Nasrat et al 1986). Furthermore, a reduction in the total number of striatal DAergic receptor binding sites was found in the 14 day old nicotine-exposed male but not female pups (data not shown).

It is possible that an increase in the basal rate of DA formation in the striatal region is a compensatory mechanism secondary to a decrease in the number of postsynaptic DA receptors and an increase in the rate of DA turnover in the nicotine-exposed pups.

However, the lack of biochemical effect of nicotine on the striatal region of female pups suggests that other central DAergic systems may also be altered by the continuous administration of nicotine to account for the increased hyperactivity in these pups. If nicotine modifies the normal development of DAergic neurons, a deficit in DAergic neuronal activity may reduce the modulatory role of DA on central excitatory noradrenergic activity, resulting in hyperactivity (Denson et al 1975; Shaywitz et al 1976; Fung 1988). Alternatively, it is possible that nicotine may have an effect on the nucleus accumbens, an important DAergic system involved in motor functions (Jackson et al 1975; Iversen & Koob 1977).

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