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Short communication

Nicotine exposure during pregnancy is a factor which influences serotonin transporter density in the rat brain

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Abstract

We examined the effects of nicotine exposure during pregnancy on serotonin transporter (SERT) expression in the brain. Nicotine (6 mg/kg/day) was administered to pregnant rats via subcutaneous injections or infusion pumps. Irrespective of the route of administration, nicotine increased SERT density in the forebrain on postnatal day 22, but not in the other brain regions. Our results suggest that nicotine use by pregnant women might be an environmental factor influencing SERT expression in their children.

Keywords: Nicotine; 5-HT (5-hydroxytryptamine, serotonin) transporter; Paroxetine; Pregnancy; Smoking

1. Introduction

The uptake of serotonin (5-hydroxytryptamine, 5-HT) from the synaptic cleft by 5-HT transporter (SERT) proteins, which are expressed on presynaptic nerve terminals, is a major mechanism for terminating the activity of the transmitter. Alterations in SERT density have been reported in mental disorders (Arango et al., 1995; Crow et al., 1984; Heinz et al., 1998; Laruelle et al., 1993; Perry et al., 1983). Although the regulation of SERT expression has been extensively investigated in animal studies, it does not seem likely that SERT is regulated by the amount of substrate, the 5-HT molecule (Yeghiayan et al., 1997), or by exposure to antagonists (Dean et al., 1997; Dewar et al., 1993), in contrast to typical membrane receptors. Recent findings from studies of chronic cocaine treatment (Cunningham et al., 1992), olfactory bulbectomy (Grecksch et al., 1997) or food restriction (Zhou et al., 1996) suggest that an altered SERT density is involved in pathological conditions such as drug abuse, depression or malnutrition.

Physiological or environmental factors during pregnancy are important for the serotonergic system. We previously reported that prenatal nicotine exposure influenced the development of serotonergic functions (Muneoka et al., 1997b). In addition, altered SERT expression by prenatal treatment with a dopamine D1 receptor agonist (Whitaker-Azmitia et al., 1990), monoamine oxidase inhibitors (Whitaker-Azmitia et al., 1994), cocaine (McReynolds and Meyer, 1998) and dexamethasone (Muneoka et al., 1997a; Slotkin et al., 1996) has been reported. However, there have been no reports examining the effects of prenatal nicotine exposure on SERT expression.

In animal studies using rodents, a bolus injection of nicotine to animals is considered as a model of human heavy smoking, whereas administration of nicotine via infusion pumps is a model of nicotine substitution, e.g., nicotine skin patches or nicotine-containing chewing gum (Muneoka et al., 1999; Slotkin, 1992). Thus, the present study examined the effects of exposure of the fetus to nicotine administered via two different routes on SERT expression, using a [^{3}H]paroxetine binding assay. The dose regimens we used were daily nicotine doses comparable to those for human smokers who smoke two to three packages of cigarettes/day. (Lichtensteiger and Schlumpf, 1993; Slotkin, 1992).

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2. Materials and methods

2.1. Animals and treatments

Male and female Sprague–Dawley rats were mated in our laboratory. The morning on which sperm-positive smears were obtained was determined to be gestational day 0. Pregnant rats were housed individually under a 12-h light/12-h dark cycle (light on 08:00 h) with free access to food and water. Subcutaneous injections of 3 mg/kg nicotine were given twice daily (08:00 and 20:00 h), from gestational day 4 until gestational day 20 (Nicotine-INJ). Controls of the nicotine injections received 1 ml/kg saline (Control-INJ). Nicotine administration by infusion was carried out using osmotic minipumps (type2ML2, Alza) implanted on the back of the animals on gestational day 4 (Nicotine-INF). The delivery rate of the pumps was 6 mg/kg/day. Controls for the nicotine infusions were implanted with minipumps containing the vehicle (Control-INF). On postnatal day 4, litters were randomly culled to 10 pumps. One or two male rats from each litter were killed by decapitation on postnatal days 7, 15 and 22 for this study. Brains were removed and dissected into three regions, the midbrain + pons–medulla, forebrain and cerebellum (Muneoka et al., 1997b). All tissues were stored at −80°C.

2.2. [^{3}H]Paroxetine binding assay

A [^{3}H]paroxetine (18.1 Ci/mmol, NEN) binding assay was carried out by the method previously described with minor modifications (Muneoka et al., 1997a). The tissues were homogenized in 25 vol. (w/v) ice-cold 50mM Tris–HCl buffer (pH 7.7) containing 5 mM EDTA. The homogenate was centrifuged at 30 000 g for 10 min and resuspended in assay buffer (50 mM Tris–HCl, pH 7.4, 120 mM NaCl, 5 mM KCl). After the second washing step, the final pellets were suspended in 333 vol. of assay buffer for the binding assay. Six [^{3}H]paroxetine concentrations (0.05–1.0 nM) were used for saturation analyses. Non-specific binding was defined with 10 μM citalopram (kindly supplied by Lundbeck, Copenhagen). Incubation of membrane suspensions was performed at 25°C for 60 min and terminated by rapid filtration through GF/B filters (Whatman). The filter was washed three times. The radioactivity was determined by liquid scintillation spectrometry. The maximum density of the binding sites (B_{max}) and the dissociation constants (K_d) were calculated by Scatchard (1949) plots and the non-linear least-squares fitting technique with the Gauss–Newton method. The protein concentration was estimated by the method of Lowry et al. (1951)

2.3. Statistical analysis

Data are presented as means ± S.E.M. Two-way analysis of variance (ANOVA) (factors of nicotine and route) was used for statistical analysis at each time point. Statistical significance was assigned at a level of $P < 0.05$.

3. Results

3.1. Effects of prenatal nicotine exposure on [^{3}H]paroxetine binding

Two-way ANOVA indicated that prenatal nicotine exposure resulted in a significant increase in B_{max} in the forebrain on postnatal day 22 ($P > 0.05$) but not on postnatal day 7 or 15. There was no interaction between the

Table 1
Effects of prenatal nicotine on B_{max} values of [^{3}H]paroxetine binding (fmol/mg protein)

Forebrain	*n*	Postnatal day 7	*n*	Postnatal day 15	*n*	Postnatal day 22[a]
Control-INJ	4	197.2 ± 11.7	7	227.8 ± 3.4	7	260.7 ± 12.6
Nicotine-INJ	4	190.9 ± 8.1	7	227.2 ± 9.3	7	281.5 ± 5.2
Control-INF	3	215.1 ± 7.8	6	229.6 ± 3.9	7	253.1 ± 7.7
Nicotine-INF	3	216.0 ± 15.3	7	234.1 ± 2.	9	283.1 ± 1.3
Midbrain + pons–medulla					*n*	Postnatal day 22
Control-INJ					7	338.5 ± 5.2
Nicotine-INJ					7	341.1 ± 8.6
Control-INF					7	341.3 ± 10.0
Nicotine-INF					9	355.9 ± 9.9
Cerebellum					*n*	Postnatal day 22
Control-INJ					7	56.4 ± 11.6
Nicotine-INJ					7	72.7 ± 6.1
Control-INF					7	62.4 ± 4.1
Nicotine-INF					9	62.9 ± 3.9

Data are indicated as means ± S.E.M.; *n*—the number of samples.
[a] $P < 0.05$; effects of nicotine in two-way ANOVA.

Table 2
Effects of prenatal nicotine on K_d values of [^{3}H]paroxetine binding (nM)

Forebrain	*n*	Postnatal day 7	*n*	Postnatal day 15	*n*	Postnatal day 22
Control-INJ	4	0.071 ± 0.009	7	0.048 ± 0.003	7	0.042 ± 0.004
Nicotine-INJ	4	0.058 ± 0.003	7	0.040 ± 0.003	7	0.045 ± 0.003
Control-INF	3	0.076 ± 0.012	6	0.049 ± 0.003	7	0.044 ± 0.002
Nicotine-INF	3	0.063 ± 0.009	7	0.047 ± 0.003	9	0.049 ± 0.004
Midbrain + pons–medulla					*n*	Postnatal day 22
Control-INJ					7	0.044 ± 0.002
Nicotine-INJ					7	0.047 ± 0.004
Control-INF					7	0.051 ± 0.003
Nicotine-INF					9	0.044 ± 0.003
Cerebellum					*n*	Postnatal Day 22
Control-INJ					7	0.173 ± 0.040
Nicotine-INJ					7	0.181 ± 0.025
Control-INF					7	0.147 ± 0.022
Nicotine-INF					9	0.155 ± 0.025

Data are indicated as means ± S.E.M.; *n*—the number of samples.

effects of nicotine and the routes of administration. No changes in B_{max} were detected in the midbrain + pons–medulla or the cerebellum on postnatal day 22 (Table 1). No significant effect of nicotine was found on the K_d (Table 2).

3.2. Effects of the difference in the routes of drug administration on [^{3}H]paroxetine binding

Two-way ANOVA showed no significant effect of the route of drug administration on B_{max} (Table 1) or K_d (Table 2).

4. Discussion

The present data demonstrated that prenatal nicotine exposure resulted in increases in SERT density, measured with [^{3}H]paroxetine, in juvenile rats. Significant increases were found in the forebrain, which is a terminal region of 5-HT neuronal projections, but not in the midbrain + pons–medulla, which includes serotonergic cell bodies, or in the cerebellum, which receives a poor innervation. These increases in SERT density were found equally among animals treated with nicotine administered via different routes. This evidence clearly indicates that the increases in SERT density are the results of nicotine itself, and are not due to other factors such as the handling accompanying drug administration or differences in pharmacokinetics between the two routes of administration. The injection or infusion of nicotine is considered a model of heavy smoking or nicotine substitution, respectively, because subcutaneous injections of nicotine induce acute increases in blood nicotine levels, vasoconstriction and hypoxia, as found in human cigarette smokers, whereas infusions via minipumps keep blood nicotine concentrations constant in a manner similar to that of nicotine skin patches or nicotine-containing chewing gum (Slotkin, 1992). Thus, the present results suggest that maternal nicotine use during pregnancy, not only smoking but also nicotine substitution, could change the SERT density in their children.

It has been suggested that repeated injections are mild stressful manipulations which elevate circulating glucocorticoid levels in pregnant rats and to influence the serotonergic system in the offspring (Peters, 1982). In fact, our previous study showed significant differences in 5-HT turnover in the midbrain + pons–medulla between rats treated with injections and infusions prenatally (Muneoka et al., 1997b). Furthermore, an increase in SERT density by glucocorticoid administration during pregnancy has been reported (Muneoka et al., 1997a; Slotkin et al., 1996). Nevertheless, the anticipated difference in the SERT density in the midbrain + pons–medulla was not found between the injection and infusion groups in this study. Therefore, the present results do not support the speculation that stress during pregnancy influences SERT expression in the offspring.

In this study, increased SERT densities were found in juvenile rats. Recent findings suggest that 5-HT functions are important for the development of social behavior during the peripubertal period (Ferris, 2000). Interestingly, prenatal nicotine exposure induced growth retardation in the offspring after the pubertal period (Muneoka et al., 1999). Alterations of the serotoninergic system, including an altered SERT density, during the juvenile period might affect physiological and behavioral development. Although further investigation is required to examine whether the increased SERT density in the forebrain is a transient phenomenon or persist in adult or aged animals, maternal nicotine used during pregnancy should be considered a factor that influences SERT density in humans.

In conclusion, the present study demonstrated that prenatal nicotine exposure was an environmental factor which influences SERT expression in the forebrain.

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