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Prenatal nicotine exposure is associated with an increase in [^{125}I]epibatidine binding in discrete cortical regions in rats

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Abstract

Previously, it was reported that hyperactive male offspring of dams exposed to nicotine (6 mg/kg/day) during gestation had an increase in cortical α4-β2 nicotinic receptor subtype density as determined by [^{3}H]cytisine binding in tissue homogenate [Tizabi Y, Popke EJ, Rahman MA, Nespor SM, Grunberg NE. Hyperactivity induced by prenatal nicotine exposure is associated with an increase in cortical nicotinic receptors. Pharmacol, Biochem Behav 1997;58:141–6]. [^{125}I]Epibatidine labels α4β2 nicotinic receptors with higher affinity than [^{3}H]cytisine. In the present study, using quantitative autoradiography, we evaluated the effects of in-utero exposure to nicotine (9 mg/kg/day) on [^{125}I]epibatidine binding in 46 discrete brain regions of 36-day-old male offspring of Sprague–Dawley rats. This dosage of nicotine administered during pregnancy to same rats was shown to result in increased vertical activity in the male offspring [Tizabi Y, Russell LT, Nespor SM, Perry DC, Grunberg NE. Prenatal nicotine exposure: effects on locomotor activity and central [^{125}I]α-BT binding in rats. Pharmacol, Biochem Behav 2000;66:495–500]. Prenatal nicotine exposure resulted in increases in receptor densities of the somatosensory cortex (90%) and the visual cortex (107%) only. Moreover, these increases were restricted to cortical layer 1. Collectively, these results indicate that prenatal nicotine exposure affects specific nicotinic receptors in selective cortical regions of male offspring. These neurochemical effects may be responsible for some of the behavioral abnormalities seen in such offspring.

Keywords: Prenatal; Nicotine; Nicotinic receptors; [^{125}I]epibatidine; Locomotor activity; Cortex; Rat

1. Introduction

In-utero exposure to nicotine, through maternal smoking during pregnancy, may result in behavioral and cognitive impairments in the offspring [5,11,19,25]. Numerous animal studies have verified neurochemical or behavioral changes following prenatal exposure to nicotine [13,22,24,28,33,34,41,44,45]. These changes are dependent on the dosage and duration of nicotine as well as the postnatal age and sex of the offspring. The effects of nicotine are most likely mediated through nicotinic receptors. These receptors are present in fetal brain by mid-gestation and are believed to play a critical role in synaptogenesis and neuronal differentiation [1,6,16,41,46]. Various nicotinic receptor subtypes with distinct developmental, physiological, and pharmacological properties have been identified [7,16,23,39,53]. The two prominent and most extensively studied central nicotinic receptors include the α4-β2 and the homomeric α7 subtypes. These receptors can be labeled by [^{3}H]cytisine or [^{125}I]α-bungarotoxin, respectively [2,8,12]. [^{3}H]Epibatidine labels with very high affinity the receptors labeled by [^{3}H]cytisine, as well as an additional population of neuronal nicotinic receptors [17,31,32]. An iodinated analog of epibatidine, [^{125}I]epibatidine (also known as [^{125}I]IPH), demonstrates an essentially identical receptor profile to [^{3}H]epibatidine, and has proven to be an excellent radioligand for autoradiography [10].

Tizabi et al. [44] had reported that selected hyperactive male offspring of dams treated with nicotine (6 mg/kg/day throughout gestation) showed increased [^{3}H]cytisine binding in cortex. This dosage of nicotine, however, did not result in overall hyperactivity in male or female offspring [44]. Recently, we reported that male offspring of dams treated with 9 mg/kg/day nicotine throughout gestation had an elevated vertical activity when tested at postnatal days

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22–24 [45]. Although this dosage of nicotine is relatively high, all dams delivered successfully [45]. Moreover, it should be noted that marked species variations exist in the biotransformation of nicotine and that even in the same species genetic differences may contribute to large variations in nicotine pharmacokinetics and pharmacodynamics [3,9,37]. The purpose of this study was to determine whether administration of the same dose of nicotine during pregnancy may also result in altered cortical nicotinic receptors in the offspring. In addition, because in the previous study receptor measurements were carried out in membrane-rich fraction obtained through homogenization of the entire cortex, no regional selectivity could be ascribed. Hence, in this study we applied autoradiography to determine whether changes in nicotinic receptors might be restricted to specific cortical layers.

2. Methods

2.1. Animals and treatments

Timed-pregnant Sprague–Dawley rats weighing 200–230 g were purchased from Charles River Laboratories (Kingston, NY). The animals were maintained in an environmentally controlled room with a 12 h light/dark cycle (lights on at 1900), a temperature range of 22–24°C, and relative humidity of approximately 50%. Subjects were housed individually in $35.6 \times 15.2 \times 20.3$ 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).

For determination of [^{125}I]epibatidine binding, eight pregnant dams were randomly divided into two groups (four per group) to receive either nicotine dihydrochloride (9 mg/kg/day nicotine base dissolved in physiological saline) or saline via osmotic minipumps (model 2002, Alza, Palo Alto, CA). The pumps were implanted on the fourth day of gestation 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. A total of 42 male pups (22 control and 20 nicotine-exposed) were used in this study.

2.2. Brain collection

All pups were weaned on day 18 postnatally. Male littermates were housed in groups of 4–6 pups per cage. They were sacrificed by decapitation at age 36 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 [24,28,38,42,46]. 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.

2.3. Nicotinic receptor measurement

Autoradiography for [^{125}I]epibatidine was done using adaptations of published methods [10,31,32]. Frozen 20 μm sagittal sections were cut, thaw-mounted onto gelatin-coated microscope slides and stored at −70°C until use. The sections were preincubated 5 min in Tris–HCl buffer (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, then incubated with [^{125}I]epibatidine (0.4 nM) for 40 min at room temperature. [^{125}I]Epibatidine (2200 Ci/mmol, NEN lot #E85453-048B) was a generous gift from Dr. Kenneth J. Kellar. Nonspecific binding was determined in adjacent sections in the presence of 300 μM (−)nicotine hydrogen tartrate. After incubation, sections were rinsed twice for 5 min each in ice-cold buffer, then dipped briefly into distilled water. After air drying, the sections were apposed to Hyperfilm BetaMax (Amersham, Arlington Heights, IL) along with [^{125}I]standards (Amersham) for 65 h. Quantitative densitometric analysis of binding was done using the Loats Inquiry digital densitometry system (Loats Associates, Winchester, MD). Brain regions were identified by comparison to previous autoradiographic results using [^{125}I]epibatidine [10], [^{3}H]epibatidine [32], or [^{3}H]nicotine [8], which were determined using adjacent Nissl-stained sections according to the atlas of Paxinos and Watson [29]. To determine specific binding, nonspecific binding in adjacent sections (which was essentially identical to film background) was subtracted from the total binding in the equivalent anatomical region. It should be emphasized, however, that the values for binding are determined by comparison of a two-dimensional image of a tissue section, with variable quenching and consistency, to a plastic standard of known radioactivity. As a result, the binding values from the autoradiographic samples presented here are perhaps best regarded as semi-quantitative.

2.4. Data analysis

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 × region) was observed, the Newman–Keuls post-hoc test was applied to identify the specific region affected. All analysis were two-tailed and used an α of 0.05 or less to determine significance.

3. Results

3.1. Effects on [^{125}I]epibatidine binding

Table 1 presents the effects of prenatal nicotine exposure on [^{125}I]epibatidine binding in 46 discrete brain regions. A significant drug effect $F(1,1890) = 9.53$, $P < .01$ as well as interaction (treatment × region) $F(1,44) = 10.6$, $P < .01$ was observed. Post-hoc analysis revealed no changes in [^{125}I]epibatidine binding in most of the 46 examined brain regions in pups exposed prenatally to nicotine. However, binding of [^{125}I]epibatidine in nicotine-exposed animals almost doubled in two regions: layer 1 of the visual cerebral cortex (+107%; $P < .01$) and layer 1 of the somatosensory cerebral cortex (+90%; $P < .01$). Sample autoradiographic images of [^{125}I]epibatidine is shown in Fig. 1.

Table 1
Density of [^{125}I]epibatidine binding (dpm/mg tissue; means ± S.E.M.) in 46 discrete brain regions of 36-day-old male rats that were exposed to nicotine (9 mg/kg/day) or saline (control) throughout gestation

Region	Control	Nicotine
Orbital cortex, L1	304 ± 8.4	291 ± 14
Orbital cortex, L3/4	476 ± 11	472 ± 13
Orbital cortex, L6	497 ± 14	447 ± 7.8
Motor cortex, L1	293 ± 7.0	287 ± 9.0
Motor cortex, L3	448 ± 11	452 ± 11
Motor cortex, L4	445 ± 8.7	429 ± 11
Motor cortex, L6	543 ± 20	489 ± 17
Somatosensory cortex, L1	272 ± 12	517 ± 11 *
Somatosensory cortex, L3	453 ± 15	469 ± 9.7
Somatosensory cortex, L4	416 ± 13	415 ± 11
Somatosensory cortex, L6	481 ± 17	470 ± 12
Visual cortex, L1	315 ± 16	654 ± 4.4 *
Visual cortex, L3	49 ± 14	486 ± 13
Visual cortex, L4	429 ± 11	442 ± 9.9
Visual cortex, L6	492 ± 12	491 ± 11
Retrosplenial cortex, L1	337 ± 18	353 ± 20
Retrosplenial cortex, L3/4	510 ± 12	517 ± 11
Piriform cortex	248 ± 9.2	239 ± 8.6
Olfactory bulb	76.6 ± 3.6	62.7 ± 2.4
Optic chiasm	517 ± 11	493 ± 10
Optic tract	506 ± 13	467 ± 11
Nucleus accumbens	485 ± 18	435 ± 19
Caudate putamen	538 ± 13	522 ± 6.2
Lateral septum	178 ± 9.1	171 ± 6.1
Amygdala	119 ± 6.3	113 ± 7.4
Hippocampus, CA1 layer	160 ± 6.6	153 ± 6.0
Dentate gyrus	200 ± 7.0	179 ± 4.6
Subiculum	491 ± 14	491 ± 12
Parasubiculum	661 ± 21	630 ± 11
Postsubiculum	693 ± 22	637 ± 4.6
Thalamus	708 ± 22	654 ± 4.4
Dorsal lateral geniculate nucleus	742 ± 32	659 ± 2.1
Medial geniculate nucleus	681 ± 25	622 ± 5.5
Medial habenula	678 ± 26	612 ± 20
Fasiculus retroflexus	730 ± 33	640 ± 14
Interpeduncular nucleus	702 ± 25	638 ± 4.7
Superior colliculus, superior gray	711 ± 26	657 ± 1.7
Olivary pretectal nucleus	669 ± 20	616 ± 7.0
Pontine nucleus	373 ± 11	348 ± 12
Microcellular tegmental nucleus	406 ± 13	371 ± 11
Median raphe nucleus	535 ± 28	473 ± 19
Medial vestibular nucleus	434 ± 10	432 ± 14
Dorsal tegmental nucleus	530 ± 11	505 ± 11
Solitary tract nucleus	392 ± 16	384 ± 13
Area postrema	586 ± 32	575 ± 13
Cerebellum	268 ± 14	265 ± 9.1

* Binding in animals exposed to nicotine significantly greater than controls, $P < .01$.

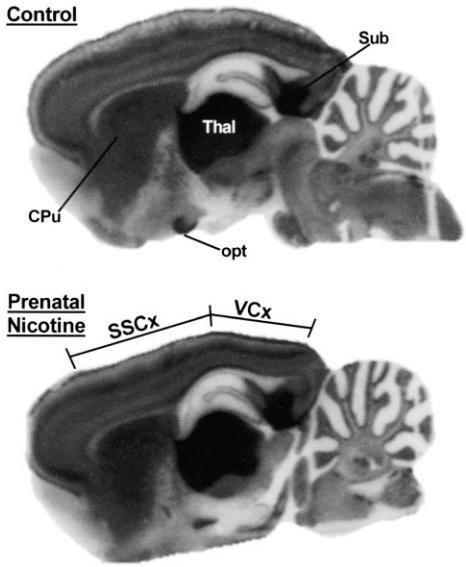

Fig. 1. Representative autoradiographic images comparing binding of [^{125}I]epibatidine (darker regions) in parasagittal brain sections of 36-day-old male offspring of dams that were treated with saline (top) or 9 mg/kg/day nicotine (bottom) during gestation. CPu (caudate putamen), opt (optic tract), SSCx (somatosensory cortex), Sub (subiculum), Thal (thalamus), and VCx (visual cortex).

4. Discussion

The results of the current study indicate that in-utero exposure to nicotine can result in an increase in [^{125}I]epibatidine binding in selective cortical regions of the male offspring. These offspring also manifest increased vertical movements [45]. Previously, it was reported that selected hyperactive male offspring of dams exposed to nicotine throughout gestation had an elevated [^{3}H]cytisine binding in the cortex [44]. Both [^{125}I]epibatidine and [^{3}H]cytisine label α4-β2 nicotinic receptor subtype, with [^{125}I]epibatidine possessing a higher affinity and less non-specific binding compared to [^{3}H]cytisine [10,32]. Epibatidine also labels an additional population of neuronal nicotinic sites [17,32], but evidence indicates that epibatidine binding in layer 1 of the cerebral cortex is largely limited to the α4-β2 subtype (Perry and Kellar, unpublished results). Collectively, these findings suggest that increases in α4-β2 nicotinic receptors in selective cortical regions of the male offspring may be responsible for some of the behavioral effects induced by prenatal exposure to nicotine.

Curiously, the observed effects were seen primarily in the sensory, rather than in the motor areas. Moreover, the changes were confined to the layer 1 of the cortex. Although the relation of these specific anatomical regions to behavioral abnormalities induced by prenatal nicotine administration remains to be elucidated, it may be that impairments in sensory processing affect the motor response. Alternatively, the observed neurochemical changes may be related to other behavioral effects of nicotine. For example, animal studies have demonstrated that prenatal nicotine exposure may also lead to cognitive impairments [22,43,52]. In humans also, fetal exposure to nicotine through cigarette smoking during pregnancy may result in learning difficulties as well as hyperactivity during childhood [5,11,19,25].

Nicotinic receptors are located primarily presynaptically where their stimulation can influence the release or activity of a variety of neurotransmitters [35,47,50]. Hence, the changes in high-affinity nicotinic receptor binding in layer 1 of the cortex may indicate a change in functionality of these receptors to effectively modulate the neurotransmitter release at the nerve terminals. In addition, these changes may also be occurring in postsynaptic nicotinic receptors that have been shown to be involved in fast acetylcholine-mediated synaptic transmission in the sensory cortex [18]. Intra-uterine exposure to nicotine results in up-regulation of nicotinic receptors in fetal as well as in postnatal brains [15,42,44,46]. An increase in nicotinic receptors following chronic nicotine administration is believed to reflect receptor desensitization [30,48,50,51]. However, it remains to be determined whether the increases in the nicotinic receptors observed in this study reflect functional desensitization or may represent a compensatory mechanism with functional receptors.

It is of relevance to note that exposure to the same dose of nicotine in-utero resulted in a reduction (22–29%) in [^{125}I]αBT binding in the hippocampal and thalamic regions (e.g. hippocampal CA1, dentate gyrus, and medial geniculate nucleus) of the male offspring [45]. A decrease in [^{125}I]αBT binding, which selectively labels the α7 nicotinic receptor subtype, following prenatal nicotine exposure is most likely due to neuronal or synaptic losses [36,41]. Thus, it may be that a decrease in a specific nicotinic receptor subtype (e.g. α7) in a discrete brain region or circuitry (e.g. hippocampus or thalamus) following intra-uterine exposure to nicotine results in a compensatory increase in another nicotinic receptor subtype (e.g. α4-β2) in another discrete brain region (e.g. cortex). In this regard, the importance of the hippocampus and cortex and their interactions in cognitive functions and gating of information is well documented [14,27]. A role for thalamo-cortical and cortico-thalamic circuitry in sensory processing and behavioral abnormalities is likewise well recognized [4,20,26,40]. Alternatively, prenatal nicotine exposure may independently result in neuronal loss in one region and functional changes in specific nicotinic receptor subtype(s) in another region. Regardless of the specific mechanism(s) involved, however, it may be suggested that prenatal nicotine exposure results in an imbalance in central nicotinic cholinergic systems that leads to behavioral abnormalities such as hyperactivity, impulsiveness, or inattention in offspring. Therefore, stimulation or normalization of the nicotinic system by administration of a nicotinic agonist may lead to reversal of some of the behavioral abnormalities precipitated by a nicotinic imbalance. In this regard it is of interest to note that some symptoms of adult ADHD are alleviated by administration of nicotine [21] or ABT-418, a nicotinic analog [49].

In summary, prenatal nicotine exposure results in an increase in α4-β2 nicotinic receptors in selective cortical regions of the male offspring. These neurochemical effects may be responsible for some of the behavioral abnormalities seen in such offspring.

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