Perinatal Nicotine Exposure Eliminates Peak in Nicotinic Acetylcholine Receptor Response in Adolescent Rats

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ABSTRACT

Maternal smoking is a risk factor associated with nicotine abuse, so the effect of perinatal nicotine exposure was studied on the responsiveness to nicotine across adolescence in the rat. Pregnant Sprague-Dawley rats were implanted with s.c. Alzet osmotic minipumps delivering nicotine (L-nicotine hydrogen tartrate, 2 mg/kg/day free base) or vehicle (0.9% saline) on gestational day 7. There was no effect of nicotine on dam weight gain, food consumption, or water consumption or on the number of live pups or weights at the time of birth. Pups were cross-fostered to obtain the following prenatal/postnatal exposure groups: control/control, nicotine/nicotine, nicotine/control, and control/nicotine. On postnatal days 28, 35, 49, and 63, nicotine-stimulated ⁸⁶Rb⁺ efflux was measured in synaptosomes prepared from the frontal cortex, hippocampus, striatum (STR), and thalamus (THL), using a previously developed method. Significant effects of treatment and concentration were detected in all four brain regions, and significant effects of age were observed in the STR and THL. Significant interactions of age and treatment were observed in each of the four brain regions. Nicotine-stimulated ⁸⁶Rb⁺ efflux peaked during adolescence in control rats. However, perinatal exposure to nicotine eliminated this peak during adolescence. These results are consistent with recent behavioral and receptor binding results from other laboratories and are the first direct evidence at the cellular level that the nicotinic acetylcholine receptor response varies during adolescence and is affected by perinatal nicotine exposure.

Nicotine is highly addictive and the most abused substance among adolescents (National Institute on Drug Abuse, 2004). Various studies suggest that the high smoking prevalence among adolescents is due to social demands (Simons-Morton, 2004), peer and parental smoking behavior (Flay et al., 1994), and maternal smoking during pregnancy (Kandel et al., 1994; Weissman et al., 1999; Buka et al., 2003; Cornelius et al., 2005; Porath and Fried, 2005; O’Callaghan et al., 2006). Offspring of mothers who smoked while pregnant were twice as likely to develop nicotine dependence than offspring of mothers who did not smoke while they were pregnant (Buka et al., 2003). The focus on adolescent nicotine use is easy to understand because the greatest likelihood for nicotine addiction occurs when cigarette smoking begins early in adolescence (Chassin et al., 1990; Taioli and Wynder, 1991; Breslau and Peterson, 1996; Kandel et al., 1997; DiFranza et al., 1998; Eissenberg and Balster, 2000; Pomerleau and Pomerleau, 2000; Breslau et al., 2001). Because exposure to nicotine in utero may predispose the offspring to seek nicotine during adolescence, leading to habitual smoking into adulthood, attention has been drawn to the effects of perinatal nicotine exposure on the developing brain.

In animal studies, prenatal exposure to nicotine has been demonstrated to produce long-lasting effects on behavior, including modification of responses to nicotine in the adolescent rat (Shacka et al., 1997; Abreu-Villaca et al., 2004; Vaglenova et al., 2004). Most often, subjects exposed to nicotine in utero or in the early neonatal period exhibit subsequent increased nicotinic acetylcholine receptor (nAChR) binding (Tizabi et al., 1997; Miao et al., 1998; Tizabi and Perry, 2000). Thus, there is every reason to believe that perinatal nicotine exposure up-regulates the functional response to nicotine. This study tests the hypotheses that

ABBREVIATIONS: nAChR, nicotinic acetylcholine receptor; PND, postnatal day; CTX, frontal cortex; HIP, hippocampus; STR, striatum; THL, thalamus; GD, gestational day; MI, mortality index; ANOVA, analysis of variance; AUC, area under the curve.
nAChRs in the brains of adolescents are particularly responsive to nicotine, and perinatal exposure to nicotine further enhances the functional response of nAChRs to nicotine in the adolescent rat brain.

The function of nAChRs of control rats and rats exposed to nicotine perinatally was studied at four specific ages across adolescence, postnatal days (PNDs) 28, 35, 49, and 63. The $^{86}$Rb$^+$ efflux superfusion assay of Marks et al. (1993) was used to measure the $n$AChR response to nicotine stimulation in synaptosomes prepared from male and female rats. Four different brain areas were selected for study, the frontal cortex (CTX), hippocampus (HIP), striatum (STR), and thalamus (THL). In the dissection used in this study, the “thalamus” contained hypothalamic nuclei and the mesencephalon, as well as thalamic nuclei. All four of these regions exhibit $[3H]$nicotine binding and nicotine-stimulated $^{86}$Rb$^+$ efflux, and the THL, in addition to containing an exceptionally high density of $[3H]$nicotine binding sites, contains the ventral tegmental area, an area considered important for reward and in addiction. The function of nAChRs, as measured by $^{86}$Rb$^+$ efflux in response to nicotine, peaked during midadolescence in all four brain regions, whereas perinatal (prenatal, postnatal, or pre- and postnatal) nicotine exposure eliminated this peak in $n$AChR function, supporting the first hypothesis but not the second hypothesis.

Materials and Methods

Materials. Rubidium-86 ($^{86}$Rb$^+$) was purchased from PerkinElmer Life Sciences (Boston, MA). (−) Nicotine hydrogen tartrate salt, tetrodotoxin, NaCl, MgSO$_4$, KCl, CaCl$_2$, and sucrose were purchased from Sigma Chemical Co. (St. Louis, MO). CsCl was purchased from VWR (Baltimore, MD) and HEPES hemisodium salt from Roche Diagnostic Corporation (Indianapolis, IN).

Animals. All studies were performed in compliance with the Institutional Animal Care and Use Committee of Virginia Commonwealth University. Male Sprague-Dawley rats (70+ days of age; Harlan Laboratories, Indianapolis, IN) were housed together with nulliparous female Sprague-Dawley rats (70+ days of age; Harlan Laboratories) until successful mating, as determined by the presence of a seminal plug. This day was designated as gestational day (GD) 0. Pregnant rats were separated into control and nicotine groups and housed individually in plastic cages with hardwood chip bedding. Rats were allowed free access to water and food (LabDiet Rodent Chow 5001; PMI Nutrition International, Richmond, IN) and were housed under a 12-h light/12-h dark cycle in a temperature- and humidity-controlled room.

On GD 7, the dams were implanted s.c. under isoflurane anesthesia with 28-day osmotic minipumps (Alzet 2ML4; Durect, Cupertino, CA) delivering nicotine (2 mg/kg free base/day) or 0.9% saline. Our laboratory has found previously that this dosing regimen produces nicotine plasma levels in the pregnant rat similar to those of humans smoking two packs of cigarettes a day without compromising maternal weight gain or food and water intake (Shacka et al., 1997). Maternal weight gain and food and water consumption were recorded daily. Within 24 h of delivery, all litters were sexed, weighed, and culled to 10 pups, keeping equal numbers of males and females where possible. Litters of 8 to 10 were retained intact. Litters of less than eight were weighed but excluded from further study. Pups were cross-fostered at this time to obtain the following prenatal/postnatal exposure groups: control/control (C), nicotine/nicotine (N), nicotine/control (NC), and control/nicotine (CN). The early postnatal period in the rat is analogous to the 3rd trimester of a human pregnancy. It has been demonstrated that pups nursing dams implanted with osmotic minipumps delivering nicotine achieve blood nicotine levels similar to those of their dams (Chen et al., 2005). Pups were weaned on PND 21 and housed in same-sex groups, and the uteri of the dams were examined to determine the number of implantation sites. Mortality index (MI = (number of fetal resorptions + number of stillbirths)/number of implantation sites) was calculated for the control and nicotine-treated groups.

Determination of $^{86}$Rb Efflux. Pups were allowed to mature to PNDs 28, 35, 49, or 63, at which time they were rapidly decapitated, and the brains were removed. These ages were selected because they span a period in the rat analogous to adolescence (Spear, 2000). No more than one male and one female from each litter were studied at each age. The cerebral cortex was pooled, and underlying HIP and STR were removed intact. The CTX was separated for use in the assay. The cerebellum was removed, and the brain stem posterior to the inferior colliculus was removed. Brain tissue rostral to the ante- rior commissure and the remaining cortex were discarded. The remaining block of tissue, which also contained the mesencephalon and the hypothalamus as well as the thalamus, was designated as THL.

Dissected tissue was placed in 10 volumes of cold 0.32 M isotonic sucrose with 5 mM HEPES hemisodium salt, pH 7.4. Synaptosomes were prepared, and the $^{86}$Rb efflux assay was performed according to Marks et al. (1993). A 25-μl aliquot of synaptosomal suspension from each brain region was incubated for 30 min with 10 μl of load buffer containing approximately 4 μCl $^{86}$Rb. Synaptosomes were then filtered onto Gelman AE glass fiber filters (Gelman Sciences, Ann Arbor, MI) under a gentle vacuum. The filters were then rinsed with 500 μl of load buffer and transferred to a polypropylene platform and superfused with perfusion buffer (25 mM HEPES hemisodium salt, 135 mM NaCl, 5 mM CsCl, 1.5 mM KCl, 2 mM CaCl$_2$, 1 mM MgSO$_4$, 1 g/l bovine serum albumin, and 50 nM tetrodotoxin, pH 7.4) for 6 min. Filters were then stimulated for 1 min with one of two concentrations of nicotine (10 and 30 μM, prepared in perfusion buffer), followed by a 3-min wash of perfusion buffer. Each sample was assayed in duplicate. Twelve-second fractions of effluent were collected and counted for 1 min each using a gamma counter (Wallac Wizard 3 1480 Automatic Gamma Counter; PerkinElmer, Shelton, CT). The magnitude of the $^{86}$Rb efflux response was calculated based on the increase in counts above the baseline after stimulation of the tissue with nicotine. Data were calculated as fractional release (counts per minute/total counts per minute loaded on filter) for each fraction collected.

Data Analysis. Maternal data (weight gain and food and water intake) were analyzed by two-way repeated measures (gestational day as repeated measure × treatment) analysis of variance (ANOVA). One-way ANOVA was used to analyze litter variables and mortality index data. Offspring weight data were analyzed by three- way ANOVA (sex × age × treatment). In the $^{86}$Rb efflux studies, the baseline was calculated for each rat by fitting to an exponential equation the fractional release in fractions immediately preceding and following the peak. The area under the curve (AUC) of each peak was calculated by the trapezoidal rule using the calculated baseline. AUCs were analyzed by four-way (treatment × sex × age × concentration) ANOVA, and treatment groups were compared with control by use of the Dunnett test. The Tukey-Kramer test was used to determine statistically significant differences between AUCs at different ages within each treatment group. $p < 0.05$ was considered to be statistically significant.

Results

Two-way (treatment × day) ANOVA determined no overall effect of nicotine on dam weight ($p = 0.35$), food consumption ($p = 0.88$), or water consumption ($p = 0.69$), shown in Fig. 1. Litter characteristics are displayed in Table 1. Although there was a strong trend for an increase in MI in the nicotine-exposed group, the difference failed to reach statistical significance ($p = 0.0540$). No significant differences were found.
in number of pups born live ($p = 0.1177$) or litter weights ($p = 0.1771$). There was also no effect of gestational nicotine exposure on the female: male ratio compared with the control group ($p = 0.2778$). Weights of our subjects were analyzed on the day of testing (days 28, 35, 49, and 63) by three-way (sex $\times$ age $\times$ treatment) ANOVA (Table 2). As expected, significant effects of sex [$F_{(1,121)} = 397.9, p < 0.0001$] and age [$F_{(3,121)} = 1118.3, p < 0.0001$] and a significant interaction of sex and age [$F_{(3,121)} = 74.2, p < 0.0001$] were observed on weight, such that the weight difference between males and females increased with increasing age. A significant interaction of age and treatment [$F_{(9,121)} = 2.3, p = 0.0210$] was detected. Rats exposed to nicotine both pre- and postnatally weighed significantly less than controls on PND 28 ($p < 0.05$, Dunnett’s test). This was the only effect of nicotine observed on weight.

The AUCs of nicotine-stimulated $^{86}$Rb$^+$ efflux were analyzed by three-way (treatment $\times$ age $\times$ sex) for each brain region (Fig. 2). As expected, significant effects of concentration were observed in the CTX [$F_{(1,274)} = 10.13, p = 0.0016$], HIP [$F_{(1,275)} = 4.33, p = 0.0383$], STR [$F_{(1,275)} = 20.90, p < 0.0001$], and THL [$F_{(1,274)} = 6.13, p = 0.0139$], where 30 $\mu$M nicotine elicited a larger response than 10 $\mu$M from the nAChRs. Significant effects of treatment were observed in the CTX [$F_{(3,274)} = 5.43, p = 0.0012$], HIP [$F_{(3,274)} = 7.01, p = 0.0001$], STR [$F_{(3,275)} = 8.46, p < 0.0001$], and THL [$F_{(3,274)} = 6.69, p = 0.0002$]. Significant effects of age were observed in only two brain regions, STR [$F_{(3,275)} = 3.48, p = 0.0165$] and THL [$F_{(3,274)} = 5.27, p = 0.0015$]. Most importantly, however, a significant treatment $\times$ age interaction was observed in all brain regions examined, CTX [$F_{(9,274)} = 3.51, p = 0.0044$], HIP [$F_{(9,275)} = 2.88, p = 0.0029$], STR [$F_{(9,275)} = 3.62, p < 0.0003$], and THL [$F_{(9,274)} = 3.14, p = 0.0013$]. In the control group, nicotine-stimulated $^{86}$Rb$^+$ efflux peaked during adolescence in each of the brain regions studied (Fig. 2). Nicotine-stimulated $^{86}$Rb$^+$ efflux was significantly larger on PNDs 35 and 49 than 63 in the CTX and THL and significantly larger on PND 35 than 63 in the HIP ($p < 0.05$, Tukey-Kramer test). In the STR, nicotine-stimulated $^{86}$Rb$^+$ efflux was significantly larger on PNDs 35 and 49 than on PNDs 28 and 63 ($p < 0.05$, Tukey-Kramer test). However, the magnitude of nicotine-stimulated $^{86}$Rb$^+$ efflux remained relatively constant across adolescence in each of the three groups of animals exposed to nicotine perinatally (i.e., N/N, N/C, or C/N). In none of these three exposure groups did nicotine-stimulated $^{86}$Rb$^+$ efflux differ significantly among postnatal ages (Fig. 2). Furthermore, the $^{86}$Rb$^+$ efflux response to nicotine in each of these groups was significantly smaller than the response in the C/C rats during adolescence. On PND 35, nicotine-stimulated $^{86}$Rb$^+$ efflux in the CTX, HIP, STR, and THL was significantly less than that of the C/C group in all exposure groups exposed to nicotine perinatally (Fig. 2, $p < 0.05$, Dunnett test). On PND 49, nicotine-stimulated $^{86}$Rb$^+$ efflux in the CTX and STR of the N/N and N/C groups was significantly less than that of C/C (Fig. 2, $p < 0.05$, Dunnett test). Furthermore, on PND 49, nicotine-stimulated $^{86}$Rb$^+$ efflux was significantly less than C/C in the HIP of the group exposed to nicotine both pre- and postnatally and in the THL of the group exposed to nicotine only prenatally (Fig. 2, $p < 0.05$, Dunnett test). In addition, nicotine-stimulated $^{86}$Rb$^+$ efflux in the CTX of rats exposed to nicotine only postnatally was significantly less than C/C rats on PND 28 (Fig. 2, $p < 0.05$, Dunnett test). Only in two brain regions, the CTX and THL, did a nicotine-exposed group have a larger $^{86}$Rb$^+$ efflux response to nicotine. On PND 63, rats exposed to nicotine both pre- and postnatally had significantly larger nicotine-stimulated $^{86}$Rb$^+$ efflux in the CTX and THL than C/C (Fig. 2, $p < 0.05$, Dunnett test). No effects or interactions with sex were observed in any brain region studied.

**Discussion**

Nicotine-stimulated $^{86}$Rb$^+$ efflux peaks during midadolescence in the rat, and perinatal nicotine exposure eliminates that peak. Behavioral studies have indicated that adolescent rats are more sensitive than adults to the effects of nicotine (Vastola et al., 2002; Adriani et al., 2003; Levin et al., 2003; Belluzzi et al., 2004), consistent with the observation that the greatest likelihood for nicotine addiction occurs when cigarette smoking begins early in adolescence (Chassin et al., 1990; Taioli and Wynder, 1991; Breslau and Peterson, 1996; Kandel et al., 1997; DiFranza et al., 2000; Eissenberg and Balster, 2000; Pomerleau and Pomerleau, 2000; Breslau et al., 2001). Furthermore, $[^3]$H]cytosine binding is significantly greater in the hippocampus and cortex of adolescent than adult rats (Trauth et al., 1999). The nicotine-stimulated $^{86}$Rb$^+$ efflux findings provide the first direct evidence at the cellular level that nAChR response peaks during adoles-
nescence. It is intriguing that perinatal exposure to nicotine completely eliminates the peak in nAChR response during midadolescence because the majority of clinical studies report that prenatal exposure to nicotine increases smoking behavior (Kandel et al., 1994; Weissman et al., 1999; Cornelius et al., 2005; Porath and Fried, 2005; O’Callaghan et al., 2006).

Several behavioral paradigms indicate that adolescent rats

### TABLE 1
Effect of prenatal nicotine exposure on litter variables

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Litter Weight</th>
<th>Live Pups</th>
<th>Males</th>
<th>Females</th>
<th>Female:Male</th>
<th>MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>86.4 ± 6.5</td>
<td>12.6 ± 1.1</td>
<td>6.0 ± 0.5</td>
<td>6.6 ± 0.8</td>
<td>1.2 ± 0.2</td>
<td>0.17 ± 0.06</td>
</tr>
<tr>
<td>Nicotine</td>
<td>13</td>
<td>69.8 ± 9.1</td>
<td>9.8 ± 1.2</td>
<td>5.3 ± 0.8</td>
<td>4.5 ± 0.7</td>
<td>0.9 ± 0.1</td>
<td>0.37 ± 0.07</td>
</tr>
</tbody>
</table>

*a* Data are expressed as mean ± S.E.M.

*b* Number of litters.

*c* MI = (number of fetal resorptions + number of stillbirths)/(number of implantation sites).

### TABLE 2
Effect of perinatal nicotine exposure on weight

<table>
<thead>
<tr>
<th>Treatment</th>
<th>28d</th>
<th>35</th>
<th>49</th>
<th>63</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control/control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>96.5 ± 5.8</td>
<td>155.0 ± 3.1</td>
<td>241.2 ± 13.4</td>
<td>298.7 ± 16.6</td>
</tr>
<tr>
<td>Female</td>
<td>90.0 ± 6.1</td>
<td>132.7 ± 7.2</td>
<td>164.7 ± 4.7</td>
<td>222.0 ± 4.6</td>
</tr>
<tr>
<td>Nicotine/nicotine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>81.6 ± 2.6</td>
<td>133.8 ± 3.8</td>
<td>244.4 ± 6.4</td>
<td>326.5 ± 7.9</td>
</tr>
<tr>
<td>Female</td>
<td>76.4 ± 1.5</td>
<td>118.1 ± 4.3</td>
<td>183.5 ± 4.1</td>
<td>218.4 ± 3.1</td>
</tr>
<tr>
<td>Nicotine/control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>95.0 ± 5.0</td>
<td>143.2 ± 5.4</td>
<td>240.2 ± 8.1</td>
<td>329.2 ± 2.9</td>
</tr>
<tr>
<td>Female</td>
<td>79.0 ± 3.5</td>
<td>126.7 ± 8.4</td>
<td>163.7 ± 10.0</td>
<td>224.4 ± 5.5</td>
</tr>
<tr>
<td>Control/nicotine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>90.0 ± 5.4</td>
<td>142.8 ± 5.1</td>
<td>241.1 ± 5.8</td>
<td>314.6 ± 2.4</td>
</tr>
<tr>
<td>Female</td>
<td>84.0 ± 3.5</td>
<td>118.0 ± 5.6</td>
<td>178.4 ± 2.7</td>
<td>214.6 ± 4.9</td>
</tr>
</tbody>
</table>

*a* Weight expressed in grams (mean ± S.E.M.).

*b* PND.
respond differently to nicotine than do adults. Levin et al. (2003) found that female adolescent rats acutely self-administer more nicotine than do adult females. Furthermore, the amount of nicotine self-administered remains greater in the adolescent-onset group after they have reached adulthood. Likewise, Adriani et al. (2003) demonstrated that periaudolescent exposure of rats to nicotine increases adult nicotine self-administration over that of rats exposed in the postadolescent period. Using a conditioned place preference paradigm, Vastola et al. (2002) found that adolescent rats develop nicotine place preference, whereas adults do not. Belluzzi et al. (2004), on the other hand, reported that male “early” adolescent rats exhibit conditioned place preference to nicotine, whereas “late” adolescent and adult rats do not. The ontogeny of nicotine sensitivity in these behavioral studies does not exactly correspond to the pattern of responsiveness to nicotine across age in the $^{86}$Rb$^+$ efflux studies. In fact, they do not even exhibit the same timeline within the same behavioral paradigms (compare Vastola et al. and Belluzzi et al.). It must be pointed out that although all of these studies were performed on the same strain, Sprague-Dawley, the rats in each study were from different suppliers. To directly compare behavior and nicotine-stimulated $^{86}$Rb$^+$ efflux, such studies should be performed with rats from the same supplier, if not within the same individual rats. However, it should suffice to say that behavioral measures of nicotine responsiveness and cellular measures of nicotine responsiveness peak in the periaudolescent period.

The basis of the large $^{86}$Rb$^+$ response to nicotine in the adolescent brain is unknown. Studies of nAChR ontogeny have focused upon the expression of these receptors during adolescence. The amount of nicotine self-administered remains greater in the adolescent-onset group after they have reached adulthood. Likewise, Adriani et al. (2003) demonstrated that periaudolescent exposure of rats to nicotine increases adult nicotine self-administration over that of rats exposed in the postadolescent period. Using a conditioned place preference paradigm, Vastola et al. (2002) found that adolescent rats develop nicotine place preference, whereas adults do not. Belluzzi et al. (2004), on the other hand, reported that male “early” adolescent rats exhibit conditioned place preference to nicotine, whereas “late” adolescent and adult rats do not. The ontogeny of nicotine sensitivity in these behavioral studies does not exactly correspond to the pattern of responsiveness to nicotine across age in the $^{86}$Rb$^+$ efflux studies. In fact, they do not even exhibit the same timeline within the same behavioral paradigms (compare Vastola et al. and Belluzzi et al.). It must be pointed out that although all of these studies were performed on the same strain, Sprague-Dawley, the rats in each study were from different suppliers. To directly compare behavior and nicotine-stimulated $^{86}$Rb$^+$ efflux, such studies should be performed with rats from the same supplier, if not within the same individual rats. However, it should suffice to say that behavioral measures of nicotine responsiveness and cellular measures of nicotine responsiveness peak in the periaudolescent period.

The basis of the large $^{86}$Rb$^+$ response to nicotine in the adolescent brain is unknown. Studies of nAChR ontogeny have focused upon the expression of these receptors during the prenatal or early postnatal period or before and after, but not during, adolescence. A larger number of neurons expressing nAChRs or larger numbers of nAChRs expressed per neuron could explain the greater response to nicotine during adolescence. Because the $^{86}$Rb$^+$ efflux assay is thought to reflect mainly $\alpha 4\beta 2^*$ nAChR activity (Marks et al., 1993), another explanation could be the expression of proportionately more of this receptor subtype by the adolescent brain. Yet another explanation for the larger response to nicotine in the nicotine-naive adolescent may involve differences in nAChR desensitization or recovery from nAChR desensitization. Whatever the mechanism involved, the enhanced effects of nicotine on nAChR function as reflected in this study’s nicotine-stimulated $^{86}$Rb$^+$ efflux may explain why adolescents are so vulnerable to the addictive effects of nicotine. Because adolescence marks the final period of neurodevelopment (Spear, 2000), it is a particularly susceptible time for exposure to any drug, including nicotine, to cause long-lasting changes in neuronal function.

The elimination of the peak in nicotine responsiveness during adolescence appears to involve a direct effect of nicotine on the developing brain during the perinatal period. High-affinity $[^{3}H]$nicotine binding appears in the fetal rat brain as early as GD 12 (Naef et al., 1992). Message for $\alpha_3$, $\alpha_4$, $\alpha_7$, and $\beta 2$ nAChR subunits are all expressed in the brain prenatally (Broide et al., 1995; Ostermann et al., 1995; Zoli et al., 1995; Winzer-Serhan and Leslie, 1997; Adams et al., 2002). Nicotine crosses the placental barrier so that it can directly affect the fetal brain (Luck et al., 1985; Hellström-Lindahl and Nordberg, 2002). The effects of perinatal nicotine exposure on nicotine-stimulated $^{86}$Rb$^+$ efflux do not appear to occur indirectly through changes in maternal behavior or nutrition. Nicotine exposure did not affect maternal nutritional status; no changes in maternal weight gain or food and water intake were observed, as has also been reported by Vaglenova et al. (2004). Furthermore, pups fostered to control dams similarly exhibited a loss of the adolescent peak in nicotine-stimulated $^{86}$Rb$^+$ efflux. The cross-fostering procedure indicates that the effect of nicotine on the nAChRs occurs at least partly in the perinatal period, as it mattered little whether the pups were exposed to nicotine prenatally or postnatally, or both.

The observation that perinatal nicotine exposure reduced the $^{86}$Rb$^+$ efflux response during adolescence is not without precedent. Abreu-Villaça et al. (2004) reported that rats exposed to nicotine prenatally fail to up-regulate $[^{3}H]$cytisine binding in the midbrain that is otherwise observed in adolescent rats treated with nicotine throughout adolescence. Seidler et al. (1992) reported that prenatal exposure to nicotine eliminated nicotine-stimulated release of norepinephrine in 30-day-old rats. Especially pertinent to the present results, Chen et al. (2005) reported a significant reduction in $^{125}$I-epibatidine binding in tissue punches from multiple brain areas of adolescent rats exposed to nicotine gestationally. This may explain the loss of the midadolescence peak in nicotine-stimulated $^{86}$Rb$^+$ efflux in rats exposed to nicotine perinatally. The effects of perinatal nicotine exposure may be especially long-lasting because it has been reported recently that neonatal nicotine exposure impairs the actions of nicotine on auditory learning in adult rats (Liang et al., 2006).

A pharmacokinetic explanation for the effect of perinatal nicotine exposure on the actions of nicotine on nAChRs can be excluded because the $^{86}$Rb$^+$ efflux assay is conducted in vitro. The decreased response to nicotine following perinatal nicotine exposure could be attributed to a change in either nAChR function or numbers. Explanations for the reduced $^{125}$I-epibatidine binding provided by Chen et al. (2005) include reduced expression of nAChR mRNA and, hence, reduced receptor synthesis, as well as loss of neurons on which nAChRs are located. Support for the latter is provided by the studies of Abreu-Villaça et al. (2004), which suggest that neuronal numbers are reduced after prenatal nicotine exposure. However, further studies need to be performed to confirm whether neurons lost as a result of prenatal nicotine exposure are nAChR-containing neurons.

It is somewhat puzzling that perinatal nicotine exposure in the rat, which is similar to prenatal nicotine exposure in the human, reduces the response to nicotine in the adolescent, when it has been commonly associated with an increased risk for smoking. One could speculate that adolescents with a reduced response to nicotine will smoke more frequently to experience the same rewarding properties, resulting in more stimulus-response associations, a stronger association between smoking cues and smoking, and nicotine dependence and habitual smoking into adulthood. Another possibility is that these adolescents may smoke to compensate for cognitive deficits resulting from in utero exposure to nicotine. A recent study reports that the smoking adolescent offspring of smokers experience greater impairment in visuospatial memory during tobacco withdrawal than do the smoking offspring of nonsmokers (Jacobsen et al., 2006). Yet another possibility is that non-$\alpha 4\beta 2$ nAChR subtypes, as opposed to $\alpha 4\beta 2$ nAChR subtypes, are not reduced, or are even in-
creased, in number or function by prenatal nicotine exposure, so that nicotine’s action at these receptors is not reduced in human adolescents prenatally exposed to nicotine. An interesting observation is that there was an increase in nAChR responsiveness to nicotine in the CTX and THL at 63 days. Hence, prenatal exposure could actually increase smoking behavior by making the young adult more sensitive to nicotine; however, older subjects must be studied to determine whether this increased responsiveness is maintained.

Eighty-five percent of smokers begin smoking before they are 18, and twice as many daily smokers begin smoking at 12 years of age as those who begin smoking at 21 years of age (United States Department of Health and Human Services, 1994). The finding that the magnitude of nicotine-stimulated $38^{6}$Rb$^+$ efflux, a measure of nAChR function, peaks during adolescence in the rat provides a physiological basis for this phenomenon. Furthermore, it has been found that perinatal exposure to nicotine leads to long-term changes in nAChR function. Therefore, it is imperative to isolate the critical time when exposure to nicotine is disruptive to development so that intervention and prevention programs for pregnant smokers can be set in place to deter smoking behavior that may be detrimental to the development of their children.

References


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