

Maternal Vaccination Against Nicotine Reduces Nicotine Distribution to Fetal Brain in Rats

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ABSTRACT

Cigarette smoking during pregnancy is associated with a variety of adverse fetal outcomes. Nicotine is a likely contributor to these adverse effects, with fetal brain as one target organ. Vaccination of adult male rats against nicotine has been shown to reduce nicotine distribution to the brain. The current study examined whether vaccination of female rats before pregnancy would reduce the distribution to fetal brain of a single nicotine dose administered during gestation. Female rats immunized with a nicotine conjugate vaccine received a single dose of nicotine 0.03 mg/kg i.v. on gestational day 16 to 22. Five minutes later, vaccinated rats had substantially higher bound and lower unbound serum nicotine concentration and lower brain nicotine concentration than controls. Fetal brain nicotine concentration was reduced by 43% in vaccinated rats, com-

parable to the reduction in the maternal brain nicotine concentration. The whole-fetus nicotine concentration was not altered by vaccination. A similar experiment was performed in which pregnant rats were passively immunized with rabbit nicotine-specific IgG 7 or 21 mg/kg just before nicotine dosing. The effects of passive immunization on nicotine distribution in the mother were IgG dose-related and the higher dose reduced nicotine distribution to fetal brain by 60%. These data suggest that vaccine effects on nicotine distribution to serum and brain are similar in pregnant female rats to those previously reported in adult males. Vaccination of female rats before pregnancy, or passive immunization during pregnancy, can reduce the exposure of fetal brain to a single dose of maternally administered nicotine.

Cigarette smoking during pregnancy is strongly associated with a variety of adverse outcomes including premature delivery, low birth weight, increased neonatal mortality, and sudden infant death syndrome (U.S. Department of Health and Human Services, 1990; Stratton et al., 2001). An emerging literature suggests that smoking during pregnancy is also associated with adverse developmental effects in children and young adults, including attention deficit hyperactivity disorder (ADHD) (Milberger et al., 1998), conduct disorder (Wakschlag et al., 1997), an increased risk of tobacco dependence (Cornelius et al., 2000), and cognitive impairment (Stratton et al., 2001). Despite these recognized adverse effects, up to 25% of pregnant women smoke throughout their pregnancy (Windsor et al., 2000).

Animal data strongly suggest that nicotine is a teratogen and is one mediator of the adverse effects of smoking on fetal outcomes (Slotkin, 1998; Ernst et al., 2001). While other tobacco or tobacco smoke toxins such as carbon monoxide

may contribute to these adverse outcomes, various physiologic, neurochemical, and behavioral effects on the fetus have been found following exposure of rats or mice to clinically relevant doses of nicotine during pregnancy (Slotkin et al., 1987; Levin et al., 1993; Newman et al., 1999; Trauth et al., 1999). Some of these effects are dose-related, suggesting that a reduction in nicotine exposure to the fetus or fetal brain (the presumed target for behavioral or cognitive sequelae) might reduce these adverse outcomes (Slotkin et al., 1997; Newman et al., 1999; Fewell et al., 2001).

Immunization of adult male rats with a nicotine conjugate vaccine has been shown to substantially reduce nicotine distribution to the brain, and has been studied as a potential treatment for nicotine dependence (Pentel et al., 2000). Vaccination elicits the production of high-affinity nicotine-specific antibodies that bind and sequester nicotine in serum and reduce the unbound nicotine concentration (Hieda et al., 1999). Antibodies are excluded from the brain by the blood-brain barrier owing to their large size (Bradbury and Lightman, 1990), so that unbound nicotine can enter the brain but antibody-bound nicotine cannot. Acting in this manner, vaccination has been found to reduce nicotine distribution to the brain and to block or attenuate a variety of nicotine-induced

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ABBREVIATIONS: Nic-IgG, nicotine-specific IgG; ELISA, enzyme-linked immunosorbent assay; ANOVA, analysis of variance.

behaviors including locomotor activation, the discriminative stimulus properties of nicotine, the development of nicotine dependence, and the relief of nicotine abstinence signs by nicotine (Pentel et al., 2000; Malin et al., 2001, 2002; Malin, 2002).

Vaccination of rats, or passive immunization of rats with rabbit nicotine-specific IgG, reduces nicotine distribution to the brain by up to 65% in the first few minutes after a single i.v. nicotine bolus dose of 0.03 mg/kg, equivalent on a weight basis to the nicotine absorbed from two cigarettes by a smoker (Hieda et al., 2000; Pentel et al., 2000). Preliminary data suggest that vaccination reduces nicotine distribution to other organs as well, although to varying extents (Satoskar et al., 2002). Vaccination might, therefore, also act in an analogous manner and reduce nicotine distribution to the fetus when nicotine is administered to the mother during pregnancy. However, the fetus differs from other organs in humans and rats in that IgG is actively transported across the placenta from mother to fetus (Laliberte et al., 1984; Zhang et al., 1988; Simister and Story, 1997). Thus it is also possible that the transfer of maternal antibody to the fetus could serve to increase nicotine delivery to the fetus and aggravate the adverse effects of maternal nicotine exposure.

The effects of maternal vaccination on nicotine distribution to the fetus are of interest for two reasons. First, several nicotine vaccines aimed at the treatment of tobacco dependence have entered phase I clinical trials. No data are available regarding the safety of such vaccines during pregnancy. Second, if vaccination reduces nicotine exposure to the fetus, it would be of interest to study whether this effect is large enough to improve fetal outcomes. In the current study, the effects of vaccination on the distribution of nicotine were evaluated in near-term pregnant rats to determine whether vaccination alters nicotine distribution to the fetus, and in particular to fetal brain. Passive immunization with nicotine-specific IgG was also studied because it allows control of the antibody dose.

Materials and Methods

Drugs and Reagents. (-)-Nicotine bitartrate, (-)-cotinine, (-)-methyl-³H-nicotine 70 Ci/mmol, and goat anti-IgG-peroxidase conjugates were obtained from Sigma-Aldrich (St. Louis, MO). Internal standards for the nicotine/cotinine assay were a gift from Dr. Peyton Jacob. Nicotine was administered to rats as nicotine bitartrate, but all doses and measured concentrations are expressed as the base.

Nicotine Vaccine. The hapten *trans*-3'-aminomethylnicotine was prepared as previously described (Pentel et al., 2000) and conjugated at the 3' position to the carrier protein recombinant *Pseudomonas aeruginosa* exoprotein A through a succinic acid linker (Fatom et al., 1993) to form the complete immunogen. This vaccine, as well as carrier proteins for the immunization of control groups, was supplied by Nabi (Boca Raton, FL).

Vaccination of Rats. Rats were immunized with nicotine vaccine 25 µg i.p. in complete Freund's adjuvant on day 0, and boosted with 25 µg of immunogen in incomplete Freund's adjuvant on days 21 and 42. Control animals were similarly immunized with carrier protein alone. Rats were allowed to mate starting after day 49, when nicotine-specific antibody titers were expected to be highest. Serum nicotine-specific antibody titers were measured by ELISA from blood obtained just before nicotine administration. All vaccinated rats were included in the protocol regardless of their serum nicotine-specific antibody titer.

Production of Nicotine-Specific IgG (Nic-IgG) in Rabbits.

New Zealand white rabbits were immunized with 100 µg of nicotine immunogen s.c. in complete Freund's adjuvant on day 0, and boosted with 100 µg of immunogen s.c. in incomplete Freund's adjuvant on days 21 and 42. Rabbits were bled weekly to obtain serum and boosted as needed to restore antibody levels. Immune rabbit serum was purified on a Protein G column and dissolved in phosphate-buffered saline at a concentration of 50 mg/ml total IgG for administration to rats (Pentel et al., 2000). The purified IgG contained 4.7% nicotine-specific IgG (Nic-IgG). All Nic-IgG doses are expressed as the weight of the Nic-IgG fraction. Control IgG consisted of human nonspecific IgG (Sandoglobulin; Novartis Pharmaceuticals, East Hanover, NJ) dissolved in phosphate-buffered saline at a concentration of 50 mg/ml. Human IgG does not bind nicotine and has been previously shown to have no effect on nicotine distribution (Pentel et al., 2000).

Antibody Characterization. The affinity and binding capacity of nicotine-specific antibody in the serum of vaccinated rats or in the rabbit IgG doses used for passive immunization were measured by radioimmunoassay (Muller, 1983). The percentage of nicotine-specific IgG in the passively administered IgG was calculated as the ratio of the binding capacity measured by radioimmunoassay (converted to grams per liter using a molecular weight for IgG of 150 kD and two binding sites per molecule) and the total protein concentration. Serum nicotine-specific IgG titers were measured by ELISA (Pentel et al., 2000) using anti-rat IgG-peroxidase as the detecting antibody for vaccinated rats, and anti-rabbit IgG-peroxidase for passively immunized rats. ELISA titers were calculated as the dilution of serum producing 50% of maximal absorbance (ED₅₀) and are expressed as the reciprocal of this dilution. Antibodies elicited by the nicotine vaccine used in this study have been previously shown to have low cross-reactivity with the major nicotine metabolites (2.7% with cotinine, <1% with nicotine-*N*-oxide) and negligible (<1%) cross-reactivity with acetylcholine, the endogenous ligand of nicotinic receptors (Pentel et al., 2000).

Nicotine-specific antibody titers of fetal tissue were measured in the same manner as serum using homogenized tissues. Potential interference of fetal tissue homogenate with this assay was first tested by spiking immune serum with nonimmune tissue homogenate. Optical density was not affected by the addition of homogenate. Nonspecific binding of fetal tissue homogenate to the polyglutamate-nicotine coating antigen was also measured by plating immune tissue homogenates (from vaccinated rats) in wells containing polyglutamate alone (rather than polyglutamate-nicotine hapten conjugate) as the coating antigen. Nonspecific binding was not detected.

Effects of Vaccination on the Distribution of Nicotine. Female Sprague-Dawley rats were housed individually with ad libitum access to food and water. Rats were vaccinated as described above with an initial dose of nicotine vaccine on day 0 and booster doses on days 21 and 42. Control rats were immunized with carrier protein alone. Mating with a proven male breeder was attempted beginning one week after the final dose of vaccine. Because mating was not always initially successful, rats became pregnant between 7 and 63 days after their last booster dose of vaccine (mean of 21 days). On gestational day 16 to 22, six rats immunized with nicotine vaccine and seven control rats were anesthetized with droperidol/fentanyl i.m., a jugular vein cannula was placed, and nicotine 0.03 mg/kg containing 10 µCi of [³H]nicotine was administered over 20 s via the jugular cannula. Five minutes after nicotine dosing rats were decapitated, and maternal trunk blood, maternal brain, fetal brain, and the remainder of each fetus were removed for assay.

Effects of Passive Immunization with Nic-IgG on Nicotine Distribution. Pregnant female Sprague-Dawley rats were obtained from Harlan (Indianapolis, IN) on gestational day 15 (the gestational period for the rat is 22 days) and housed individually with ad libitum access to food and water. On gestational day 16 to 22, rats were anesthetized with droperidol-fentanyl i.m. and cannulas placed in the right jugular and left femoral veins. Groups of seven rats re-

ceived Nic-IgG 21 mg/kg, Nic-IgG 7 mg/kg, or control IgG 21 mg/kg over 30 min in 1.4 ml phosphate-buffered saline via the femoral cannula. Thirty minutes later, all rats received nicotine 0.03 mg/kg containing 10 μ Ci of [3 H]nicotine administered over 20 s via the jugular cannula. Five minutes after nicotine dosing rats were decapitated, and maternal trunk blood, maternal brain, fetal brain, and the remainder of each fetus were removed for assay. Fetal blood was obtained by cardiac puncture but not all litters provided sufficient fetal serum for assay.

Nicotine Assay. Nicotine concentration in serum or homogenized brain was calculated from the specific activity of the radiolabeled nicotine (Hieda et al., 1999). Radiolabel assay was used rather than gas or liquid chromatography because of the limited sample size available for assay of some measures, such as fetal serum nicotine concentration or protein binding. The use of radiolabel is acceptable for estimating nicotine concentration 5 min after nicotine dosing because only negligible concentrations of nicotine metabolites are formed in the rat over this interval (Hieda et al., 1999) and results are comparable to those obtained by gas chromatography (unpublished data). Radioactivity was determined by liquid scintillation counting. All fetal serum or brain tissue from a single litter was combined for analysis so that each dam yielded a single value for fetal serum or brain. Brain nicotine concentration was not corrected for organ blood content because sufficient fetal blood was not obtained for all litters to measure the fetal blood nicotine concentration. However, the error from this omission is likely quite small because brain blood content is low (1.5–3.7% in adult rats) and correction for blood content does not appreciably change the estimate of brain nicotine concentration in this organ (Pentel et al., 1987; Khor et al., 1991). All nicotine concentrations are expressed as weight of the base.

Protein Binding. Protein binding of nicotine in maternal serum was measured by equilibrium dialysis for 4 h at 37°C using 0.3 ml serum in Teflon cells (Pentel and Keyler, 1988). Protein binding was measured in maternal serum from the group passively immunized with Nic-IgG 21 mg/kg and the corresponding control group. Serum from one control rat was excluded from analysis because of sample contamination.

Statistical Analysis. Assignment of rats to treatment groups was randomized. Nicotine concentrations in serum or tissues and other maternal or fetal features (Table 1) were compared in vaccinated rats by two-tailed *t* tests, and in passively immunized rats by one-way ANOVA with Tukey's post-test. Fetal weight was analyzed in two ways: by comparing the mean of the individual fetal weights for each litter and by comparing the total fetal weight for each litter. The ratio of whole fetus to maternal serum nicotine concentration was compared among actively or passively immunized rats (excluding control groups) by one-way ANOVA. The relationship of maternal serum antibody titer to fetal antibody titer or fetal brain nicotine concentration was analyzed by linear regression.

TABLE 1

Maternal and fetal characteristics at the time nicotine was administered
Values are the mean \pm S.D.

	Maternal Weight	Number of Fetuses	Individual Fetal Weight	Total Litter Weight	Gestational Date
	<i>g</i>		<i>g</i>	<i>g</i>	<i>day</i>
Vaccinated					
Control	317 \pm 21	13.7 \pm 2.8	2.8 \pm 2.1	35.3 \pm 25.8	18.8 \pm 1.7
Nicotine vaccine	337 \pm 24	10.9 \pm 2.7	3.1 \pm 2.2	32.1 \pm 21.6	18.0 \pm 1.6
Passively immunized					
Control	299 \pm 21	11.4 \pm 2.6	2.8 \pm 1.9	29.6 \pm 18.4	18.1 \pm 2.7
Nic-IgG 7 mg/kg	413 \pm 15***	13.4 \pm 2.5	1.7 \pm 0.5	22.8 \pm 6.4	18.3 \pm 0.5
Nic-IgG 21 mg/kg	304 \pm 21	12.6 \pm 2.0	3.3 \pm 2.1	40.3 \pm 26.3	18.9 \pm 2.7

*** *p* < 0.001 compared with the control and Nic-IgG 21 mg/kg groups.

Results

Vaccination

General. There were no significant differences between the vaccinated and control groups in maternal weight, gestational dates, number of fetuses, individual fetal weight (mean value per litter), or total fetal weight per litter (Table 1).

Maternal Antibody Affinity and Titer. The K_d for nicotine of pooled serum from vaccinated rats was 40 nM. The serum antibody binding capacity of nicotine was 1.5×10^{-6} binding sites/liter, corresponding to a nicotine-specific IgG concentration of 0.12 g/l. The mean maternal serum nicotine-specific antibody titer in vaccinated rats ranged from 6,480 to 85,700. The two lowest titers were in the two rats studied at the longest intervals since their last vaccine booster dose (4 and 9 weeks).

Maternal Nicotine Concentrations (Fig. 1). Maternal serum nicotine concentrations were substantially higher in vaccinated rats compared with controls (*p* = 0.016), and brain nicotine concentrations were lower (*p* = 0.049). The mean serum nicotine concentration was increased by 430% in vaccinated rats and the brain nicotine concentration was reduced by 42%.

Fetal Nicotine Concentrations and Antibody Titers. Fetal brain nicotine concentration was also significantly reduced in vaccinated rats (*p* = 0.047), with the 44% reduction being similar to that of maternal brain. The whole-fetus nicotine concentration was not significantly altered by vaccination (Fig. 1). There was no significant correlation between the maternal serum and whole-fetus nicotine-specific antibody titers of individual animals (r^2 = 0.01, *p* = 0.87) or between the maternal serum antibody titer and fetal brain nicotine concentration (r^2 = 0.01, *p* = 0.85).

Passive Immunization

General. There were no significant differences among groups in gestational dates, number of fetuses per litter, individual fetal weight, or total fetal weight per litter (Table 1). Maternal weight was higher in the Nic-IgG 7 mg/kg group than the other two groups (*p* < 0.01), but there was no difference in maternal weight between the Nic-IgG 21 mg/kg group and controls.

Maternal Antibody Affinity and Titers. The K_d for nicotine of rabbit IgG used for passive immunization was 7 nM. Nicotine-specific antibody titers in rats receiving Nic-IgG were 123,000 \pm 10,300 for the 7 mg/kg group and

TABLE 2

Protein binding of nicotine in maternal serum

Data are from rats passively immunized with Nic-IgG 21 mg/kg or control IgG. The increase in total nicotine concentration in the Nic-IgG group was due to a substantial increase in protein binding of nicotine. The unbound nicotine concentration was significantly lower in the Nic-IgG group than in controls.

	[Nicotine] Total <i>ng/ml</i>	[Nicotine] Unbound <i>ng/ml</i>	Nicotine Bound <i>%</i>
Control	15.9 ± 4.2	14.0 ± 2.3	10.5 ± 10.4
Nic-IgG	441.0 ± 36**	7.6 ± 0.8**	98.3 ± 0.2**

** $p < 0.001$.

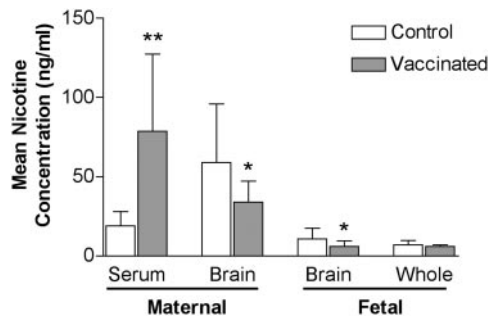


Fig. 1. Effects of vaccination on maternal and fetal nicotine concentrations (mean ± S.D.). Rats were vaccinated before pregnancy, and received 0.03 mg/kg nicotine i.v. on gestational day 16 to 22. Serum and tissues were sampled 5 min later. In the vaccinated rats the maternal serum nicotine concentration was higher and the brain nicotine concentration lower than in controls. Fetal brain nicotine concentration was reduced in vaccinated rats to the same extent as the nicotine concentration in maternal brain, while the whole-fetus nicotine concentration was not altered by vaccination. *, $p < 0.05$; **, $p < 0.01$.

190,000 ± 38,000 (mean ± S.D.) for the 21 mg/kg group. Note that these titers cannot be directly compared with those obtained with vaccination because different detecting antibodies were used to detect rat IgG (for vaccinated rats) or rabbit IgG (for passively immunized rats). To allow comparison of the relative amounts of IgG transferred from mother to fetus in the vaccination and passive immunization protocols, the ratio of the whole-fetus antibody titer to the maternal serum antibody titer was calculated. This ratio was approximately 100-fold lower in passively immunized rats compared with vaccinated rats, indicating markedly less transfer of the passively infused rabbit IgG to the fetus than the endogenous IgG resulting from maternal vaccination.

Maternal Nicotine Concentrations (Fig. 2). The effects of passive immunization on maternal nicotine distribution were dose-related (ANOVA $p < 0.001$ for differences among groups in both maternal serum and maternal brain nicotine concentrations). The mean maternal serum nicotine concentration was 15.3 times higher in the 21 mg/kg Nic-IgG group than in controls ($p < 0.001$) and 8.1 times higher in the 7 mg/kg Nic-IgG group ($p < 0.001$). This higher concentration of nicotine in the Nic-IgG groups was due to a substantial increase in protein binding from 10.5 ± 10.4% in controls to 98.3 ± 0.2% in the Nic-IgG 21 mg/kg group (Table 2). The concentration of unbound nicotine in serum was 47% lower in the 21 mg/kg Nic-IgG group compared with controls ($p < 0.001$). The maternal brain nicotine concentration in the 21 mg/kg Nic-IgG group was 82% lower than in controls ($p < 0.001$), and in the 7 mg/kg Nic-IgG group was 66% lower than in controls ($p < 0.01$).

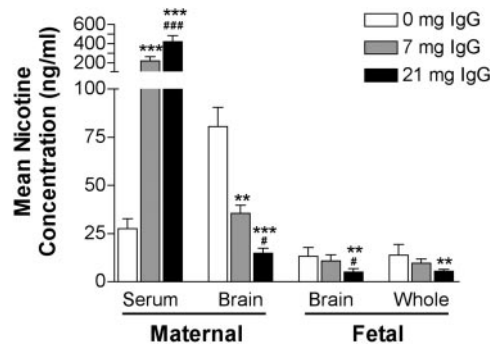


Fig. 2. Effects of passive immunization with rabbit Nic-IgG on maternal and fetal nicotine concentrations (mean ± S.D.). Female rats received Nic-IgG (7 or 21 mg/kg) or control IgG i.v. on day 16 to 22 of gestation followed 30 min later by nicotine 0.03 mg/kg i.v. Passive immunization increased nicotine retention in maternal serum and reduced nicotine distribution to maternal brain. Passive immunization at the higher dose also reduced nicotine distribution to fetal brain. Unlike vaccination, passive immunization at the higher dose also reduced nicotine distribution to the whole fetus. **, $p < 0.01$; ***, $p < 0.001$ compared with controls; #, $p < 0.05$; ###, $p < 0.001$ compared with the 7 mg/kg group

Fetal Nicotine Concentrations and Antibody Titers.

The effects of passive immunization on fetal nicotine distribution were dose-related (ANOVA $p < 0.001$ for differences among groups in fetal brain nicotine concentrations and $p < 0.01$ for whole fetus nicotine concentrations). The nicotine concentration in fetal brain was 63% lower in the 21 mg/kg Nic-IgG group than in controls ($p < 0.001$), but was not significantly lower in the Nic-IgG 7 mg/kg group. In contrast to vaccination, passive immunization at the 21 mg/kg Nic-IgG dose also reduced nicotine distribution to the whole fetus by 61% ($p < 0.001$). Whole-fetus nicotine concentrations in rats receiving the 7 mg/kg Nic-IgG dose were not significantly different from those of controls. Sufficient fetal serum for nicotine quantitation was obtained from three control litters and four litters in the 7 mg/kg Nic-IgG group. Results showed a trend toward a reduction in fetal serum nicotine concentration: control 8.9 ± 1.4, Nic-IgG 4.1 ± 0.6 ng/ml. Because of the small number of samples, statistical analysis was not performed.

To compare antibody transfer from mother to fetus between vaccinated and passively immunized groups, the ratio of whole-fetus to maternal serum antibody titers was compared by ANOVA. Control groups were not included in this analysis because they had no maternal nicotine-specific antibody (titers represented the assay background). This ratio was approximately 100-fold higher in the vaccine group (0.019 ± 0.01) compared with either passively immunized group (Nic-IgG 7 mg/kg group 0.00025 ± 0.00006, Nic-IgG 21 mg/kg group 0.00018 ± 0.00012; $p < 0.01$), indicating greater

transfer of antibody from mother to fetus with vaccination compared with passive immunization.

Discussion

The main findings of this study were that 1) immunization of pregnant rats altered maternal nicotine distribution in a manner similar to that previously reported for adult males, with increased binding and sequestration of nicotine in serum and decreased distribution of nicotine to brain; and 2) immunization of pregnant rats reduced the distribution of a single nicotine dose to fetal brain. Both vaccination of rats before mating and passive immunization after mating were effective. These data suggest that immunization could be of interest as a means of reducing the transfer of maternal nicotine to the fetus.

The rat was used as a model for vaccination effects on fetal nicotine distribution for three reasons. First, considerable data are already available regarding the effects of vaccination on nicotine distribution in the rat. Second, nicotine is readily transferred from mother to fetus in both humans and rats. Nicotine crosses the perfused human placenta from the maternal to the fetal side with little placental metabolism (Pastrakuljic et al., 1998). Nicotine concentrations in human placentas, amniotic fluid, and umbilical veins at delivery all exceed concurrent nicotine concentrations in the maternal serum of smokers (Luck et al., 1985). Limited data in rats show substantial nicotine concentrations in a variety of tissues, including brain, within 5 min of a maternal nicotine dose (Mosier and Jansons, 1972). Third, the placentas of pregnant rats and humans share several important features, including a discoid anatomy and the absence of maternal tissue interposed between maternal blood and fetal tissues (Ruckebusch et al., 1999). In addition, both rat and human placentas have a transport system for the transfer of maternal IgG to the fetus, which is absent in many other species (Laliberte et al., 1984; Zhang et al., 1988; Simister and Story, 1997). Thus, despite other differences between rat and human pregnancies (e.g., duration, litter size, and the timing of certain developmental events), the rat provides a reasonable model for a first examination of vaccine effects on fetal nicotine distribution.

Vaccination reduced nicotine distribution to maternal brain by 42%, a smaller reduction than the 60 to 65% reported in some previous studies of adult male rats (Hieda et al., 2000; Pentel et al., 2000). While gender or pregnancy status could contribute to this difference, the maternal serum antibody titers achieved in this study were also lower than in previous studies, probably because of the longer interval between the last vaccine dose and the time of study. Nevertheless, the reduction in maternal brain nicotine concentration due to vaccination was significant, as was the reduction in fetal brain nicotine concentration. No relationship was found between the antibody titer in maternal serum and the fetal brain nicotine concentration. This lack of correlation could be due in part to variability in factors such as fetal weight and litter size that were observed among pregnancies.

Vaccination has not been previously studied in female rats, but its effects on nicotine binding in serum and distribution to brain were quite similar to those previously reported in adult male rats. Protein binding in maternal serum was increased from 10% in controls to 98% after 21 mg/kg Nic-

IgG, compared with 97% after the same Nic-IgG dose in male rats (Malin et al., 2001), and 93 to 98% in rats vaccinated with the same nicotine immunogen (Satoskar et al., 2002). The nearly 50% reduction in the unbound nicotine concentration in maternal serum was also similar to reductions reported in male rats (Malin et al., 2001). Thus, changes in the serum binding of nicotine in pregnant rats, as well as its distribution to maternal brain, were very similar to those of adult male rats. Female rats or pregnant female rats may differ from males in nicotine clearance (Kyerematen et al., 1988), but such differences would not be expected to have an impact on the disposition of acutely dosed nicotine as used in this study. Such differences may prove to be more important in the setting of repeated or chronic nicotine dosing.

The 21 mg/kg Nic-IgG dose used for passive immunization was intended to exceed the estimated amount of nicotine-specific antibody present in a vaccinated rat by severalfold (Pentel and Malin, 2002). This Nic-IgG dose did produce effects larger than those achieved by vaccination in both mother and fetus, including a greater reduction in fetal brain nicotine concentration. These data suggest that higher antibody doses, whether achieved by more effective vaccination or by passive immunization, can increase the impact of immunization on the distribution of nicotine to fetal brain. It is also possible that the lower K_d of Nic-IgG for nicotine (7 nM) compared with antibodies in the serum of vaccinated rats (40 nM) contributed to the larger effect of passive immunization. Whether the 40% (with vaccination) to 60% (with passive immunization) reduction in distribution of nicotine to fetal brain seen in this study is large enough to mitigate the adverse effects of gestational nicotine exposure on fetal brain is not known. The dose-response relationship for several adverse effects of fetal nicotine exposure on brain development suggests that this magnitude of reduction could be useful (Slotkin et al., 1997; Fewell et al., 2001). Larger effects on nicotine distribution may be possible with the use of higher Nic-IgG doses.

The current study used only a single nicotine dose rather than the repeated or chronic exposure relevant to human smoking behavior. In adult male rats, vaccination remains effective in reducing nicotine distribution to brain even with repeated or chronic nicotine dosing at rates approximating heavy smoking, although less so than in the setting of a single nicotine dose (Keyler et al., 1999; Hieda et al., 2000). These quantitative relationships clearly need further study in the pregnant rat to assess the potential clinical usefulness of immunization to reduce the harmful effects of fetal nicotine exposure during pregnancy.

In contrast to vaccination, passive immunization at the 21 mg/kg Nic-IgG dose reduced nicotine distribution to the whole fetus. This may have been due to the magnitude of all effects on nicotine distribution being greater with passive immunization owing to the high Nic-IgG dose, or to the somewhat higher affinity of Nic-IgG for nicotine compared with antibodies in the serum of vaccinated rats. However, the transfer of maternal antibody to the fetus was also 100-fold higher after vaccination than after passive immunization. The lesser transfer of passively administered antibody was likely due, at least in part, to the short (30 min) interval between antibody administration and nicotine dosing, such that there was only limited time for antibody transfer to take place. The nicotine-specific antibody transferred to the fetus

in vaccinated rats may have either escorted bound nicotine into the fetus or served as a reservoir to allow the accumulation of nicotine in the fetus. As a result, the lower unbound nicotine concentrations in maternal serum of vaccinated rats compared with controls did not result in lower total nicotine transfer to the fetus. Within the fetus, however, the effects of nicotine-specific antibodies paralleled their effects in the mother, reducing nicotine distribution to fetal brain.

In summary, the effects of immunization on nicotine distribution to serum and brain in pregnant female rats were similar to those previously reported in adult male rats. In addition, both maternal vaccination and passive immunization reduced the distribution of a single nicotine dose to fetal brain. Insofar as nicotine is one of the components of tobacco responsible for adverse fetal outcomes, these data suggest that immunization may be of interest as a potential means of protecting the fetus from some of these adverse effects. Further studies to better establish the relationships among antibody concentration in serum, nicotine dose, and nicotine distribution, particularly under conditions of chronic nicotine dosing, are needed to assess the potential usefulness of this strategy. Such studies should also be useful for assessing the safety of vaccinating nonpregnant women against nicotine as a potential treatment for nicotine addiction.

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