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Reduced nicotine distribution from mother to fetal brain in rats vaccinated against nicotine: Time course and influence of nicotine dosing regimen

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Abstract

Nicotine is a teratogen in rats and possibly in humans. Vaccination against nicotine is being studied as a possible treatment for nicotine dependence. The safety of maternal vaccination against nicotine during or prior to pregnancy is not known. In this study, female rats were vaccinated and then administered acute or chronic nicotine during pregnancy at doses simulating nicotine exposure in smokers. Maternal vaccination reduced nicotine distribution to both maternal brain (44–47%) and fetal brain (17–39%) for up to 25 min after a single maternal nicotine dose administered on gestational day (GD) 20, but had a smaller effect on nicotine distribution to brain after continuous nicotine infusion. Nicotine distribution to maternal or fetal brain after repeated nicotine bolus doses was reduced immediately following an individual dose in vaccinated rats, but the chronic accumulation of nicotine in fetal brain was not altered. Nicotine distribution to whole fetus, in contrast to fetal brain, was generally not altered by vaccination. Nicotine-specific antibody concentration in fetal serum was 10% that of maternal serum, and in fetal brain was <1% of maternal serum. Although nicotine transfer to the whole fetus was not reduced by vaccination, protein binding data suggest that nicotine-specific antibody transferred from mother to fetus served to bind nicotine in fetal serum, reduce the unbound nicotine concentration, and thereby reduce nicotine distribution to fetal brain. These data comment on the safety of vaccination against nicotine during pregnancy, and suggest that vaccination may reduce the distribution of nicotine to fetal brain under some nicotine dosing conditions.
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1. Introduction

Vaccination or passive immunization (transfer of heterologous drug-specific antibody) is being studied as a potential treatment for drug addiction. Vaccination elicits the production of high affinity drug-specific antibodies which bind drug in serum and extracellular fluid, reduce drug distribution to brain and attenuate the drug's behavioral effects [1–6]. Vaccination or passive immunization has been shown to reduce nicotine, cocaine or phencyclidine-induced locomotor activation [6–8], the discriminative stimulus properties of nicotine [4,9], reinstatement of nicotine self-administration [10], and the maintenance of cocaine or methamphetamine self-administration [11,12]. Clinical trials of vaccines for cocaine and nicotine addiction have been initiated [13] (S. Winston, personal communication).

The safety profile of vaccines or passive immunization for addictive drugs is favorable. Because the elicited antibodies do not appreciably enter the brain [14], immunization circumvents the central nervous system side effects common to many other potential pharmacotherapies for drug addiction. The high degree of binding specificity of these antibodies also minimizes potential side

Abbreviations: GD, gestational day; Nic-vaccine, nicotine-vaccine

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effects outside of the central nervous system. No important adverse effects of either vaccination or passive immunization have been found to date in either animals or in early clinical trials [13,15].

While side effects of vaccination or passive immunization have not been identified, few data are available regarding the safety of immunization during or prior to pregnancy. Adverse effects of the antibody per se on the fetus are not expected [16]. However, addictive drugs may have important adverse effects on fetal viability and development. Nicotine is a teratogen in rats, and gestational exposure to nicotine in rats has been associated with altered c-fos expression [17] and neurotransmitter concentrations [18] in fetal brain, altered locomotor activity in pups [19], and altered responses to hypoxia in pups [20]. In humans, smoking during pregnancy is associated with spontaneous abortion, low birth weight, increased neonatal mortality, sudden infant death syndrome and the development of behavioral problems including conduct disorder, attention deficit hyperactivity disorder, and an increased risk of becoming a smoker in young adults [21,22]. Animal and human studies suggest adverse outcomes after gestational exposure to cocaine or methamphetamine as well [23,24]. It is therefore important to understand whether immunization alters fetal exposure to these drugs. Because fetal brain is a specific target for many of these adverse effects, the effects of immunization on drug distribution to fetal brain is of particular interest.

In a previous study, vaccination of female rats (prior to mating and pregnancy) reduced the early distribution of a single dose of nicotine administered on gestational day (GD) 16–22 to fetal brain [25]. Passive immunization of pregnant rats with heterologous nicotine-specific IgG just prior to nicotine dosing on GD16-22 was similarly effective. However, only passive immunization reduced nicotine transfer to the whole fetus. These data suggested that immunization might reduce nicotine distribution to fetal brain either by reducing total nicotine distribution to the fetus, or by altering nicotine distribution within the fetus. Because serum antibody concentrations and nicotine binding in fetal serum were not measured, the underlying mechanisms and the reasons for differences between vaccination and passive immunization remain unclear. The previous study also examined only a single nicotine dose and a single time interval (5 min) from nicotine dosing to sampling.

The current study expands upon previous data by examining the time course of vaccination effects on nicotine distribution to the fetus, and the effects of vaccination on nicotine distribution under a variety of acute and chronic nicotine dosing conditions which approximate nicotine exposure in smokers. Measures of maternal and fetal antibody concentrations and nicotine protein binding were obtained to estimate antibody transfer to the fetus and to elucidate the mechanisms by which vaccination alters nicotine distribution to fetal brain.

2. Methods and materials

2.1. Drugs and reagents

(−)-Nicotine bitartrate, (−)-cotinine, and polyvinyl pyrrolidone were obtained from Sigma (St. Louis, MO). ^{3}H-(−)-nicotine was obtained from New England Nuclear. Goat anti-rat IgG-peroxidase conjugate was obtained from Jackson Immunoresearch. Internal standards for nicotine and cotinine assay were provided by Dr. Peyton Jacob. All nicotine doses are expressed as weight of the base.

2.2. Nicotine-vaccine (Nic-vaccine)

Nicotine-vaccine was prepared by conjugating the hapten trans-3′-aminomethylnicotine at the 3′ position to the carrier protein rEPA through a succinic acid linker [6]. Antibodies produced by this vaccine in rats have been previously shown to have low cross-reactivity with the nicotine metabolites cotinine (2.7%) and nicotine-N-oxide (<1%), and with the endogenous nicotinic receptor ligand acetylcholine (<1%) [6].

2.3. Vaccination of rats

This study was approved by the Institutional Animal Care and Use Committee. Female Holtzman rats weighing 200–225 g were vaccinated prior to mating with Nic-vaccine or control-vaccine (rEPA alone) 25 μg i.p. in complete Freund's adjuvant on day 0, and in incomplete Freund's adjuvant on days 21, 42 and 63. Rats immunized with Nic-vaccine were included in these studies only if serum nicotine-specific antibody concentrations following the third or fourth vaccine dose were >30 μg/ml, and 79% of rats achieved this concentration. This serum antibody concentration (equivalent to a titer of >1:10,000) has been shown to reduce nicotine distribution to adult rat brain [26]. Vaccinated female rats were placed with male breeder rats starting 1 week after the final vaccine dose. If mating was not successful within 3 weeks, rats were again boosted with vaccine on day 84, as this is necessary to maintain high serum antibody concentrations (unpublished data). Rats were not boosted if already pregnant.

2.4. Immunologic assays

Serum and brain nicotine-specific antibody concentrations were measured by ELISA [6]. The ELISA absorbance was converted to antibody concentration using a standard curve consisting of serum samples for which nicotine-specific antibody concentrations had been determined by radioimmunoassay [27]. The between days coefficient of variation for this assay was <4% over a nicotine-specific antibody concentration range of 50–250 μg/ml, and sensitivity was 50 ng/ml. For some samples (25 min protocol), ELISA titers were measured prior to adopting this proce-

dure, and insufficient serum was available to measure concentrations as above. For these samples, ELISA titers were converted to antibody concentrations using a previously determined linear correlation between ELISA titer and antibody concentration (determined by RIA) measured on the same serum samples [14].

2.5. Mating

One week after the fourth vaccine dose, female rats were placed in male breeder cages until pregnancy was confirmed by the presence of vaginal sperm or a vaginal plug. The date of onset of pregnancy was designated GD0. For protocols 3 and 4, cannulas were placed prior to mating as detailed below. Mean weight on GD0 was 374 ± 38 g (mean $\pm$ S.D.).

2.6. Cannulations

For protocols 3 and 4 which required daily nicotine dosing, indwelling cannulas were placed prior to mating to avoid the stress of anesthesia during pregnancy. Rats were anesthetized using fentanyl/droperidol and a cannula was placed in the right jugular vein. The catheter was filled with polyvinyl pyrrolidone 2%, plugged, and exteriorized in the intrascapular region. A steel mesh shoulder harness was placed and the catheter secured to the harness to protect it during mating. After successful mating, the harness was removed. Pregnant rats were housed singly in cages with swivels and tethers to allow nicotine dosing throughout pregnancy when required [28].

2.7. Protocol 1: single nicotine dose, 5 min

The purpose of this protocol was to study nicotine distribution 5 min after a single nicotine dose administered on GD20. Groups of eight rats immunized with either Nic-vaccine or Control-vaccine were anesthetized on GD20 and a jugular vein cannula placed. Nicotine bitartrate 0.05 mg/kg (weight of the base) was administered via the catheter over 30 s. Five minutes later, rats were decapitated. Trunk blood and brain were obtained from the mother, and serum or tissue frozen for analysis. Use of trunk blood is reasonable with this protocol because arterial and venous nicotine concentrations equalize within 1 min after a bolus i.v. injection of nicotine [29]. All fetuses were rapidly removed and placed on ice. Fetal blood was obtained by cardiac puncture and pooled so that each litter yielded one serum sample. Fetal brain was removed and similarly pooled to yield one sample per litter.

2.8. Protocol 2: single nicotine dose, 25 min

This protocol was identical to the 5 min protocol except that sampling was done 25 min after the nicotine dose.

2.9. Protocol 3: continuous nicotine infusion

The purpose of this protocol was to study nicotine distribution on GD20 after continuous nicotine infusion throughout pregnancy. Eight vaccinated and eight control rats were placed in single cages on GD1 and the internal jugular catheter attached by swivel and tether to an infusion pump delivering nicotine 2 mg/kg/day. On GD20, rats were anesthetized and the nicotine infusion was terminated. The purpose of terminating the nicotine infusion was to avoid inadvertent administration of nicotine from the catheter due to bending or manipulation of the catheter during handling of the animal prior to sacrifice. After terminating the nicotine infusion, a femoral vein catheter was placed for blood sampling. Twenty five minutes after termination of the nicotine infusion, blood was obtained from the femoral catheter. The rat was then decapitated, and maternal brain, fetal serum and fetal brain were obtained as above.

2.10. Protocol 4: repeated nicotine doses

The purpose of this protocol was to study the distribution of nicotine on GD20 in rats receiving daily repeated nicotine doses. ^{3}H-nicotine was added to the final nicotine dose. This procedure permitted study of the distribution of the final nicotine dose (as radiolabeled drug) independent of the repeatedly administered nicotine (measured as unlabeled drug). Eight vaccinated and eight control rats were placed in single cages on GD1 and the internal jugular catheter attached by swivel and tether to an infusion pump delivering nicotine 0.03 mg/kg/dose over 1 s in a volume of 50 μl for 16 h/day at 14 min intervals, for a total dose of 2 mg/kg/day. The 0.03 mg/kg dose was chosen because it is well tolerated in awake rats, and the serum concentrations associated with repeated bolus doses of this size have been well characterized [28]. Dosing began at 10 p.m. each day, corresponding to the start of the lights out (active) cycle, and continued until 2 p.m. of the next day. On GD20 between 9:00 a.m. and 11:00 a.m., rats were anesthetized immediately after a nicotine dose and the infusion pump was turned off. This time of day was chosen to approximate nicotine distribution near the end of a daily dosing cycle. A femoral vein cannula was placed for blood sampling. A final nicotine dose of 0.05 mg/kg (to match the dose used in protocols 1 and 2) containing 3 μCi ^{3}H-nicotine was administered via the jugular catheter 28 min after the last programmed dose. The 28 min interval was needed to allow sufficient time for placement of the femoral vein catheter. Rats were sacrificed 25 min after the final nicotine dose and samples collected as above.

2.11. Protein binding

Nicotine binding in serum was measured by equilibrium dialysis for 4 h at 37 °C [30]. For fetal samples, the serum

from all litters within a group was pooled to obtain sufficient volume (0.7 ml) for this analysis. The unbound fraction of nicotine was calculated as the ratio of the buffer side to the serum side. The unbound nicotine concentration in serum was calculated as [total serum nicotine] × fraction unbound.

2.12. Nicotine and cotinine assay

Unlabeled nicotine and cotinine in serum or tissue was analyzed by gas chromatography with nitrogen phosphorus detection [31]. Radiolabeled nicotine was measured by scintillation counting. Whole fetus and brain were homogenized and digested in 20%, w/v of 2M NaOH overnight at 25 °C prior to extraction [29]. Maternal brain nicotine concentrations were corrected for blood content of the brain [29]. Fetal brain nicotine concentrations were not similarly corrected because fetal brain blood content data are not available.

2.13. Statistical analysis

Nicotine, cotinine and antibody concentrations in serum and tissue samples were compared between groups using unpaired, one-sided *t*-tests. One-sided comparisons were used because a previous study has shown reduced nicotine transfer to fetal brain in vaccinated rats [25]. The unit of statistical analysis for fetal data was the litter rather than individual fetuses because the volume of serum or weight of brain obtained from individual fetuses was too small for individual analysis. For equilibrium dialysis, which requires even larger volumes, serum from all litters in a group were combined into a single sample. No statistical analysis could be performed for this measure. The relationships between antibody concentrations and nicotine concentrations were analyzed using linear regression with log transformation of the nicotine concentrations to achieve the best fit.

3. Results

3.1. Protocol 1: single dose nicotine, 5 min

Most rats became pregnant within 2 weeks of their fourth vaccine dose, so that GD20 occurred 4–5 weeks after the 4th vaccine dose. The mean maternal and fetal serum nicotine-specific antibody concentrations are shown in Table 1. All rats included in this protocol had maternal serum antibody concentrations above 30 μg/ml. Maternal and fetal serum nicotine-specific antibody concentrations were highly correlated (Fig. 1, top), with a maternal/fetal concentration ratio of approximately 10:1. The concentration of nicotine-specific antibody in fetal brain (Table 1) was 6% that of fetal serum (<1% that of maternal serum) indicating limited transfer of antibody into the fetal central nervous system.

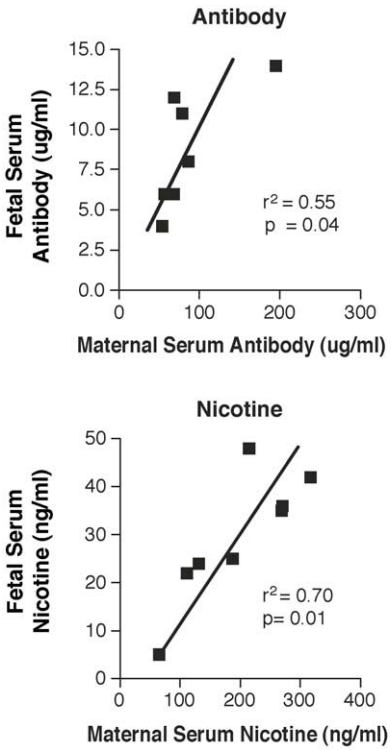

Fig. 1. Single nicotine dose, 5 min. Top: correlation between maternal and fetal serum antibody concentrations. Bottom: correlation between maternal and fetal serum nicotine concentrations. Serum was obtained from vaccinated rats 5 min after i.v. administration of nicotine 0.5 mg/kg. Fetal data represent pooled samples from all rats in a litter.

The maternal serum nicotine concentration 5 min after the nicotine dose (Fig. 2, top) was nearly 10-fold higher ($p < 0.001$) in the vaccinated rats (immunized with Nic-vaccine) than in controls (vaccinated with control-vaccine). Maternal brain nicotine concentration was reduced by 44% in the vaccine group ($p < 0.001$) compared to controls.

Table 1
Serum and brain nicotine-specific antibody concentrations in vaccinated rats, mean ± S.D., $n = 8$ per group

Protocol	Maternal serum (μg/ml)	Fetal serum (μg/ml)	Fetal brain[a] (μg/g)
Single nicotine dose, 5 min	83 ± 46	8 ± 3	0.5 ± 0.2
Single nicotine dose, 25 min	140 ± 120	10 ± 6	–
Continuous nicotine infusion	158 ± 126	12 ± 8	–
Repeated nicotine doses	277 ± 127	30 ± 32	–

The mean maternal serum antibody concentrations differed somewhat among protocols, but the maternal:fetal concentration ratios in all cases were approximately 10:1.

[a] Fetal brain concentration was measured only in the single dose, 5 min protocol.

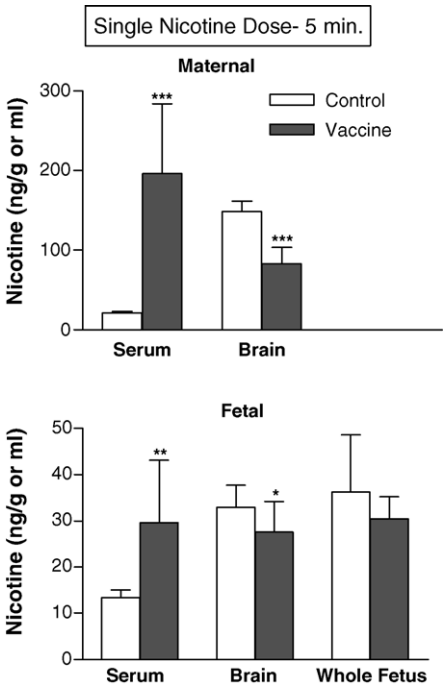

Fig. 2. Single nicotine dose, 5 min. Nicotine 0.05 mg/kg was administered to vaccinated rats on GD20 as a single i.v. bolus dose over 30 s. Tissues were obtained 5 min later. Top: Nic-vaccine increased retention of nicotine in maternal serum and reduced nicotine distribution to maternal brain. Bottom: Nic-vaccine had similar effects on the fetus, increasing retention of nicotine in fetal serum and reducing nicotine distribution to fetal brain. In contrast, nicotine distribution to the whole fetus was not altered by vaccination. Values are the mean ± S.D., $n = 8$ per group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to control group.

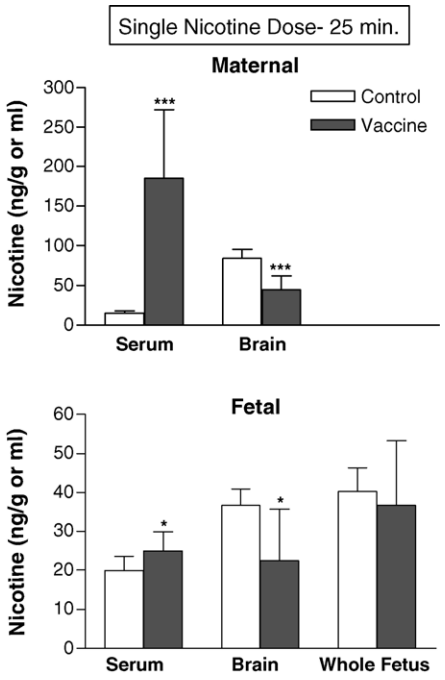

Fig. 3. Single nicotine dose, 25 min. Protocol as for Fig. 1 but tissues were obtained 25 min after the nicotine dose. Results were similar to those observed 5 min after the nicotine dose; Nic-vaccine increased retention of nicotine in maternal and fetal serum, reduced nicotine distribution to maternal and fetal brain, but did not alter nicotine transfer to the whole fetus. Values are the mean ± S.D., $n = 8$ per group. $^{*}p < 0.05$, $^{***}p < 0.001$ compared to control group.

The effects of vaccination on fetal nicotine distribution were qualitatively similar to its effects in the mother (Fig. 2, bottom). Fetal serum nicotine concentration was approximately doubled in the vaccine group ($p = 0.006$), and fetal brain nicotine concentration was minimally (17%) but significantly reduced ($p = 0.043$). In contrast, nicotine concentration in the whole fetus did not differ between groups ($p = 0.125$). There was a close correlation between maternal and fetal serum nicotine concentrations in immunized rats (Fig. 1, bottom).

3.2. Protocol 2: single nicotine dose, 25 min (Fig. 3)

Results in this protocol were overall quite similar to those in the 5 min protocol and demonstrated a sustained effect of vaccination. The mean maternal and fetal serum antibody concentrations (Table 1) were somewhat higher than in the 5 min group but were, like the 5 min group, highly correlated with each other (data not shown, $r^2 = 0.70$, $p < 0.001$). The maternal brain nicotine concentration in vaccinated rats was 47% lower ($p < 0.001$) than in controls (Fig. 3, top). The fetal brain nicotine concentration was 39% lower ($p = 0.010$) than in controls (Fig. 3, bottom). Fetal brain nicotine concentrations were lowest in rats with the highest maternal ($p = 0.030$) or fetal ($p = 0.007$) serum antibody concentrations (Fig. 4). Whole

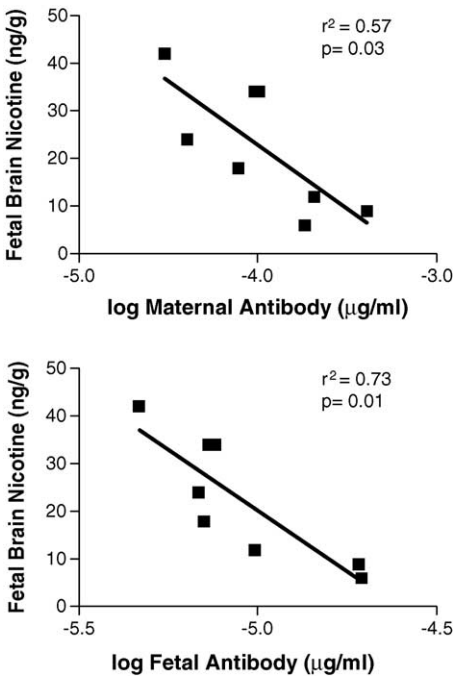

Fig. 4. Single nicotine dose, 25 min. Samples were obtained from vaccinated rats 25 min after i.v. administration of nicotine 0.5 mg/kg. Both the maternal (top) and fetal (bottom) serum antibody concentrations showed a significant correlation with the fetal brain nicotine concentration. Fetal data represent pooled samples from all rats in a litter.

fetus nicotine concentration was not altered by vaccination ($p = 0.289$).

3.3. Protocol 3: continuous nicotine infusion (Fig. 5)

The maternal serum nicotine concentration in control rats was 39 ± 8 ng/ml. The effects of vaccination on maternal and fetal nicotine concentrations were generally similar to those seen after single nicotine doses, but smaller in magnitude. The maternal brain nicotine concentration was 16% lower ($p = 0.023$) in vaccinated rats than in controls. Fetal brain nicotine concentration was reduced by 19% ($p = 0.010$) in vaccinated rats. Whole fetus nicotine concentration was not altered by vaccination ($p = 0.387$). Fetal weights did not differ between groups (4.00 ± 0.76 g versus 4.03 ± 0.98 g) Fig. 5.

3.4. Protocol 4: repeated nicotine doses

Results are presented separately for unlabeled nicotine, representing cumulative nicotine from repeated dosing (Fig. 6, left), and ^{3}H-nicotine, representing the final nicotine dose (Fig. 6, right). Effects of vaccination on nicotine distribution were generally greater for the final ^{3}H-nicotine dose than for the cumulative unlabeled dose, and similar to those found when a single dose of nicotine was administered alone (protocol 2). Fetal weights did not differ between groups (3.50 ± 0.70 g versus 3.45 ± 0.88 g).

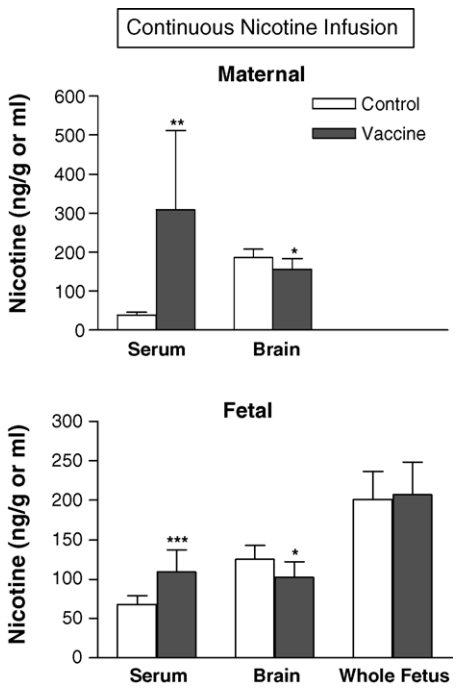

Fig. 5. Continuous nicotine infusion. Vaccinated rats were cannulated, then mated, and nicotine infused at a rate of 2 mg/kg/d starting on GD1 and continuing throughout pregnancy. Rats were anesthetized on GD20 and serum or tissue samples obtained. Nic-vaccine reduced nicotine distribution to maternal and fetal brain, but the reductions were small. Values are the mean ± S.D., $n = 8$ per group. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to control group.

3.4.1. Unlabeled nicotine

Although the unlabeled maternal serum nicotine concentration 25 min after the final nicotine dose was 12-fold higher in vaccinated than in control rats ($p = 0.001$), maternal unlabeled brain nicotine concentrations did not differ between groups ($p = 0.090$). The fetal serum unlabeled nicotine concentration was doubled in the vaccine group ($p = 0.005$) but brain concentrations ($p = 0.372$) and whole fetus concentrations ($p = 0.417$) did not differ.

3.4.2. ^{3}H-nicotine

In contrast to the unlabeled concentrations, the maternal brain ^{3}H-nicotine concentration was reduced by 41% ($p < 0.001$) and fetal brain ^{3}H-nicotine concentration was reduced by 35% in the vaccine group ($p < 0.001$). In contrast to protocols 1–3, whole fetus ^{3}H-nicotine concentration was 28% lower in the vaccine group ($p < 0.001$).

3.5. Protein binding (Table 2)

3.5.1. Nicotine

Data represent mean ± S.D. for maternal serum, but are single values for fetal serum because samples had to be pooled to provide sufficient volume for analysis. In all cases, protein binding of nicotine in serum was substantially increased in vaccinated rats compared to controls, and the unbound serum nicotine concentration was lower. Although statistical comparison was not possible for fetal samples, there was a consistent finding of higher protein binding and a lower unbound nicotine concentration in fetal serum of vaccinated rats compared to controls. Protein binding in maternal serum of vaccinated rats was in all cases higher than that in fetal serum Table 2.

3.5.2. Cotinine

Maternal serum protein binding of cotinine was increased in vaccinated rats compared to controls (repeated nicotine dose protocol: 7.8 ± 7.2 versus $0.5 \pm 0.6\%$, $p = 0.011$). However, this increase was considerably less than the increase in nicotine protein binding in vaccinated rats.

3.6. Cotinine concentrations

Maternal serum cotinine concentration was higher in vaccinated than control rats after continuous nicotine infusion (Table 3). Differences in serum cotinine concentrations in the repeated nicotine dose protocol, or in fetal serum values, were not significant. There was, however, a trend toward higher serum cotinine concentrations in all of the vaccine groups. The serum nicotine/cotinine ratios were consistently higher in the vaccine groups than in

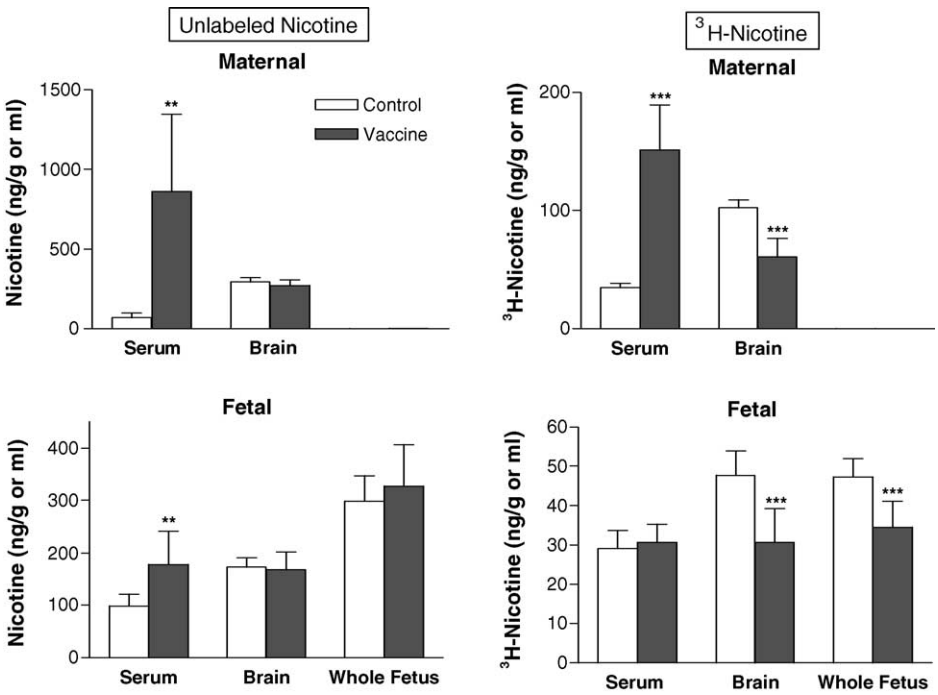

Fig. 6. Repeated nicotine doses. Vaccinated rats were cannulated, mated and nicotine administration begun on GD1 and continued throughout pregnancy. Nicotine was administered as repeated nicotine boluses of 0.03 mg/kg over 1 s every 14 min for 16 h/day (total dose 2 mg/kg/d) to approximate nicotine exposure in a smoker. On GD20, rats were anesthetized and a final nicotine dose of 0.05 mg/kg containing ^{3}H-nicotine was administered to allow differentiation of the cumulative nicotine dose (unlabeled nicotine) from that of the final nicotine dose (^{3}H-nicotine). Left panels show that the distribution of the cumulative nicotine dose to brain was not significantly altered by Nic-vaccine. Right panels show that the distribution of the final nicotine dose was altered in a manner similar to that of single nicotine doses administered alone (see Figs. 2 and 3): Nic-vaccine increased nicotine retention of the final nicotine dose in maternal and fetal serum, and reduced distribution of the final ^{3}H-nicotine dose to maternal and fetal brain. Distribution of the final dose to whole fetus was not altered by Nic-vaccine. Values are the mean ± S.D., $n = 8$ per group. $^{**}p < 0.01$, $^{***}p < 0.001$ compared to control group.

Table 2
Nicotine serum protein binding

Group	Maternal		Fetal	
	Nicotine %bound	Unbound nicotine (ng/ml)	Nicotine %bound	Unbound nicotine (ng/ml)
Single nicotine dose				
5 min				
Nic-vaccine	92 ± 4***	13 ± 5**	63	11
Control-vaccine	2 ± 4	21 ± 2	14	12
Single nicotine dose				
25 min				
Nic-vaccine	94 ± 5***	8 ± 4**	63	9
Control-vaccine	9 ± 4	14 ± 3	0	20
Repeated nicotine dose				
Unlabeled Nic				
Nic-vaccine	94 ± 2***	37 ± 7*	54	81
Control-vaccine	3 ± 3	45 ± 6	8	90
^{3}H-Nic				
Nic-vaccine	85 ± 6***	20 ± 5***	2	18
Control-vaccine	8 ± 5	32 ± 4	39	28

Values are mean ± S.D. Fetal values represent a single determination from pooled serum.
* $p < 0.05$ compared to Control-vaccine group.
** $p < 0.01$ compared to Control-vaccine group.
*** $p < 0.001$ compared to Control-vaccine group.

Table 3
Serum nicotine/cotinine concentrations and ratios in continuous nicotine infusion and repeated nicotine dosing protocols

Group	Maternal	Fetal
Continuous nicotine infusion		
Nic-vaccine		
Nicotine (ng/ml)	309 ± 203***	110 ± 27***
Cotinine (ng/ml)	481 ± 49***	402 ± 46
Nicotine/cotinine ratio	0.64	0.27
Control-vaccine		
Nicotine (ng/ml)	39 ± 8	68 ± 11
Cotinine (ng/ml)	383 ± 52	373 ± 56
Nicotine/cotinine ratio	0.10	0.18
Repeated nicotine doses		
Nic-vaccine		
Nicotine (ng/ml)	863 ± 485***	178 ± 63**
Cotinine (ng/ml)	620 ± 171	751 ± 675
Nicotine/cotinine ratio	1.4	0.23
Control-vaccine		
Nicotine (ng/ml)	68 ± 31	98 ± 22
Cotinine (ng/ml)	520 ± 110	499 ± 99
Nicotine/cotinine ratio	0.13	0.20

** $p < 0.01$ compared to control-vaccine group.
*** $p < 0.001$ compared to control-vaccine group.

controls, with the difference due primarily to higher serum nicotine concentrations in the vaccine groups.

4. Discussion

The main findings of this study are that (1) vaccination reduced nicotine distribution to fetal brain for up to 25 min after a single nicotine dose, (2) vaccine effects on nicotine distribution were greater after a single nicotine dose than after chronic nicotine dosing by either continuous infusion or repeated bolus doses, (3) the transfer of nicotine-specific antibody from mother to fetus was limited but sufficient to enhance the binding of nicotine in fetal serum and reduce nicotine distribution to fetal brain, and (4) transfer of antibody to fetal brain was negligible. These data add to our understanding of the mechanisms by which vaccination alters nicotine distribution during pregnancy, and comments on the safety of potential nicotine-vaccine use during pregnancy. Because vaccines are also being developed for cocaine, phencyclidine and methamphetamine addiction, these data may also be of more general interest.

The four nicotine dosing regimens used in this study represent a wide range of clinically relevant dosing conditions: a single i.v. bolus nicotine dose approximately equivalent to the nicotine absorbed from three cigarettes by a smoker, which simulates the rapid absorption of nicotine from a cigarette [29], and continuous nicotine infusion or repeated bolus doses to simulate chronic nicotine exposure at serum levels in the range observed in a moderate to heavy smoker [32]. While none of these regimens entirely duplicates nicotine delivery by smoking, they do provide key aspects of such exposure.

The rat is a useful model for studying drug disposition during pregnancy because rat and human placentas share anatomical features including a discoid anatomy and the absence of maternal tissue interposed between maternal blood and fetal tissue [33], and both have antibody FcRn receptors which allow transport of maternal immunoglobulin to the fetus [34–36]. A limitation of the rat model is that maturation of the rat fetus at birth is incomplete compared to humans, so that some developmental events which take place in utero in humans take place in the early neonatal period in the rat [37]. In addition, serum immunoglobulin concentrations in the human fetus are low in the first trimester but approximate those of maternal serum at delivery [35]. In contrast, we found that fetal serum nicotine-specific IgG concentrations were only 10% of maternal levels late in pregnancy. In these respects, the rat may best model early human pregnancy. While this is a potential limitation of the model, the vulnerable period for the teratogenic effects of nicotine is not well established, and data relating to all phases of pregnancy are of interest.

The effects of vaccination on maternal nicotine distribution on GD20 were remarkably similar to those previously reported in adult male rats, showing that vaccination substantially reduces the early distribution of nicotine to brain (up to 25 min), with a lesser effect on its later or steady state distribution [14,26]. The effects of vaccination on nicotine distribution to fetal brain were in general quite similar to those on maternal brain. Fetal brain nicotine concentrations were lower than in controls at 5 min after a single nicotine dose, as previously reported [25] and at 25 min after a single nicotine dose administered alone or after repeated nicotine doses. An effect of vaccination on nicotine distribution to fetal brain is further supported by the significant inverse correlation observed between maternal or fetal serum antibody concentrations and fetal brain nicotine concentrations. Vaccination had less effect on the distribution to fetal brain of chronically administered nicotine, reflecting the pattern observed in adult females in this study and previously in adult males.

It is possible that vaccination affected the distribution of chronically administered nicotine less than that of single doses because the body burden of nicotine from chronic dosing is greater. However, the robust effect of vaccination on the distribution of the final nicotine dose in the repeated dose protocol, where the final dose was administered in the presence of already very high serum nicotine concentrations, argues against this as the predominant mechanism. Moreover, vaccination has been shown to reduce nicotine distribution to brain even after a single nicotine dose of 2 mg/kg, which is 40 times the dose used in this study [38]. The current data do not further explain the mechanism, but strongly support a predominant effect of vaccination on early nicotine distribution to both mother and fetus.

The toxicologic implications of reduced nicotine distribution to fetal brain are not entirely clear. Vaccination either reduced or had no effect on nicotine transfer to fetal brain under the wide range of nicotine dosing conditions studied. Vaccination would not, therefore, be expected to increase the risk of adverse neurologic effects in the fetus, and could potentially reduce such adverse effects. It is possible that the higher fetal serum antibody concentrations seen in term human fetuses compared to rats would serve to increase total nicotine concentrations in the fetus and exposure to nicotine outside of the brain. However, the concentration of unbound, and presumably pharmacologically active, nicotine in fetal serum was not increased by vaccination, suggesting that an increase in adverse effects of nicotine outside of brain would not be expected. Studies of fetal outcomes will be needed to address these questions.

Some studies of nicotine neurotoxicity in the rat suggest that the adverse effects of gestational nicotine exposure may be greater after continuous nicotine infusion than after a single daily dose [39]. However, a single daily dose is very different from the dozens to hundreds of daily doses characteristic of smoking. The relative contributions of peak nicotine concentration and duration of nicotine exposure to the broad range of adverse neurologic effects of fetal nicotine exposure are therefore unclear.

In contrast to its effect on nicotine distribution to fetal brain, vaccination generally did not reduce nicotine distribution to the whole fetus. Distribution to whole fetus was reduced only for ^{3}H-nicotine in the repeated nicotine dose protocol, while unlabeled nicotine in this protocol and measures in all other protocols showed no difference between vaccinated and control rats. This lack of effect is likely due to the binding and sequestration of nicotine in fetal serum by nicotine-specific antibody, which allowed the antibody in fetal serum to act as a reservoir for nicotine. Although statistical comparison was not possible, the fraction of nicotine bound in fetal serum was increased and the unbound nicotine concentration decreased by vaccination in all protocols. It is likely that decreased nicotine distribution to fetal brain was primarily due to the lower unbound drug concentration in serum. Thus fetal brain nicotine exposure was reduced despite the unchanged whole body nicotine burden, and the higher total nicotine concentration in serum, in vaccinated fetuses. These data suggest that vaccination acted in the fetus in a manner entirely analogous to how it acted in the mother; binding nicotine in serum, reducing the unbound drug concentration, and reducing nicotine distribution to brain.

Vaccination has been shown to reduce nicotine distribution to adult rat brain to a greater extent than some other tissues. A contributing factor may be that antibody is largely excluded from the adult brain, so that brain antibody concentrations are substantially lower than in many other tissues [14]. In the current study, fetal brain antibody concentrations were also quite low (compared to fetal serum), indicating maturity of the fetal blood–brain barrier in this respect. This observation suggests that, as in adult rats, exclusion of antibody from fetal brain may have contributed to the observed reductions in nicotine distribution to fetal brain.

Maternal and fetal serum antibody concentrations were highly correlated, and rats with the highest serum antibody concentrations had the lowest brain nicotine concentrations. The magnitude of this effect was substantial, with a four-fold range in fetal brain nicotine concentrations as a function of serum antibody concentration. This finding suggests that strategies to enhance the antibody response to vaccination and to maximize serum antibody concentrations could substantially enhance the efficacy of vaccination in reducing fetal brain nicotine exposure. It is also possible that a vaccine which elicits antibodies with a higher affinity for nicotine would be more effective, but data specifically relating efficacy to affinity are not available.

Serum cotinine concentrations are commonly used as a measure of nicotine intake in humans, since cotinine has a longer serum elimination half-life than nicotine and is less affected by the timing of nicotine dosing over the day [40]. In addition to altering nicotine distribution, vaccination reduces nicotine clearance in the rat more than 10-fold [41], so that lower cotinine concentrations might be expected in vaccinated than in control rats. However, nicotine-specific antibodies elicited by this nicotine-vaccine also have some limited cross reactivity with cotinine (3%) and in this study vaccination increased cotinine binding in serum, potentially increasing cotinine retention in serum. There was a trend toward higher cotinine levels in vaccinated rats but this differences was significant only in maternal serum during continuous nicotine infusion. Whether serum cotinine concentrations can be used to estimate nicotine exposure in vaccinated humans will require further study because humans convert a higher percentage of a nicotine dose to cotinine than do rats. It is also possible that cotinine binding to antibody could compromise the capacity of the antibody to bind nicotine and thus reduce antibody efficacy in altering nicotine distribution. While this is possible, its importance is likely minor because vaccination of rats with this nicotine-vaccine is nevertheless clearly effective in reducing or blocking a wide range of physiological and behavioral effects of nicotine [1,4,6,9,10,42].

In summary, maternal vaccination reduced the distribution of nicotine to fetal brain for up to 25 min after a single nicotine dose administered alone or with concurrent chronic nicotine exposure at doses comparable to cigarette smoking. Although nicotine distribution to the whole fetus was generally not altered, vaccination reduced the concentration of unbound nicotine in fetal serum. These data suggest that maternal vaccination should not aggravate the adverse effects of gestational nicotine exposure. Whether reduced nicotine distribution to brain as a result of

vaccination might be sufficient to protect against the teratogenic effects of nicotine is not yet clear.

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