Biochemical Pharmacology 81 (2011) 1164–1170

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Vaccination against nicotine alters the distribution of nicotine delivered via cigarette smoke inhalation to rats

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ARTICLE INFO

Article history:
Received 11 January 2011
Received in revised form 8 February 2011
Accepted 9 February 2011
Available online 17 February 2011

Keywords:
Nicotine
Smoke
Cigarette
Antibody
Immunotherapy
Vaccine
Immunogenicity

ABSTRACT

Preclinical models of nicotine vaccine pharmacology have relied on i.v. or s.c. administration of nicotine. Models using cigarette smoke inhalation might more accurately simulate nicotine exposure in smokers. Nicotine vaccine effects were examined in rats using two cigarette smoke exposure models: a 10 min nose-only exposure (NSE) producing serum nicotine levels equivalent to the nicotine boost from 1 cigarette in a smoker, and a 2 h whole-body exposure (WBE) producing serum nicotine levels similar to those associated with regular mid-day smoking. Vaccination prior to 10 min smoke NSE reduced nicotine distribution to brain by 90%, comparable to its effect on nicotine administered i.v. Vaccination prior to 2 h smoke WBE reduced nicotine distribution to brain by 35%. The nicotine concentration in broncheoalveolar lavage (BAL) fluid obtained after 2 h WBE was increased by 230% in vaccinated rats but was also increased in rats passively immunized with a nicotine-specific monoclonal antibody, and so was likely due to transfer of antibody from serum rather than local production at the pulmonary mucosa. Nicotine-specific IgA was not detectable in BAL fluid, but titers in serum were appreciable at 21–25% of the IgG titer and could contribute to vaccine efficacy. Both vaccination and passive immunization are effective in reducing nicotine distribution to brain in rats when nicotine is delivered via inhaled cigarette smoke. These data validate results previously obtained in rodents for nicotine vaccines using i.v. or s.c. nicotine dosing and provide a quantitative method for studying aspects of nicotine exposure which are unique to cigarette smoke inhalation.

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1. Introduction

Nicotine vaccines are being studied as a treatment strategy for tobacco dependence. Immunization elicits the production of nicotine-specific antibodies which bind nicotine with high affinity and specificity in serum and extracellular fluid, reduce or slow nicotine distribution to brain, reduce nicotine clearance, and attenuate a wide variety of addiction-like behaviors in rats or mice [1]. Nicotine vaccines have entered clinical trials and several have shown preliminary evidence of efficacy for enhancing smoking cessation rates [2–4]. Nicotine vaccines were developed through investigation in rodent models of nicotine addiction, and animal work continues in an effort to better understand their mechanism of action and improve their efficacy [5].

Because immunization against nicotine is a pharmacokinetic intervention [1], it is important that animals models used to study its mechanism of action and effects model the key features of nicotine pharmacokinetics in cigarette smokers. Rodent models of nicotine exposure generally consist of nicotine administered intravenously or subcutaneously [6]. At appropriate doses, these modes of nicotine administration produce arterial and venous serum nicotine concentrations similar to those of cigarette smokers [7,8]. However they differ from the nicotine exposure of cigarette smokers in the route of absorption (inhaled vs. parenteral) and the absence of the thousands of other chemicals that are present in tobacco smoke. Tobacco components such as bicarbonate may influence the rate of nicotine absorption, and lung contains enzymes which contribute to nicotine metabolism [9,10]. In addition, pulmonary mucosa produces antibody, principally IgA, that could contribute to effects of nicotine vaccines on nicotine disposition. It is unclear to what extent these aspects of nicotine

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0006-2952/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.bcp.2011.02.004

dosing from cigarette smoke inhalation are important in understanding and modeling the use of nicotine vaccines. Inhalation of nicotine from tobacco smoke in humans allows rapid absorption from the lung into left atrial blood and results in a high initial arterial nicotine concentration which is delivered to the brain [11,12]. In smokers this is produced by discrete puffs of a cigarette inhaled over a 5–10 min period, whereas rat models generally use a single i.v. bolus dose of nicotine equivalent to the dose absorbed from 1 to 2 cigarettes because this dose produces clinically relevant serum nicotine concentrations and maintains behaviors of interest such as nicotine self-administration [6]. Subcutaneous dosing of nicotine results in absorption over 10–20 min but generally utilizes nicotine doses equivalent to 10–20 cigarettes. These models of nicotine dosing appear to model many of the clinical effects of nicotine reasonably well, but clearly differ in key respects from nicotine exposure during cigarette smoking.

Cigarette smoke exposure of rodents has been used widely for studying smoke toxicology [13,14] but its use to investigate nicotine pharmacokinetics or tobacco addiction has been quite limited [15–19], and no studies to date have used rodent smoke exposure to investigate pharmacotherapies for tobacco addiction. In the current study rats were exposed to cigarette smoke under well defined conditions modeling the smoking of 1 cigarette over 10 min or the smoking of multiple cigarettes over 2 h, as well to as i.v. nicotine. The effects of a nicotine vaccine on the distribution of nicotine to brain and the retention of nicotine in broncheoalveolar lavage fluid were assessed. Effects of passive administration with a nicotine-specific monoclonal antibody were also studied to clarify the potential role of pulmonary mucosal antibody, since vaccination may stimulate pulmonary mucosal antibody production whereas passive immunization would not.

2. Methods

2.1. Animals

Male Holtzman Sprague–Dawley rats (Harlan, Indianapolis, IN) weighing 300–325 g at the time of arrival were housed individually under a 12 h light/dark cycle and were studied during the light (inactive) cycle. Beginning 1 week after arrival, animals were restricted to 18 g/day of food to prevent them from becoming too large for the NSE restraint bottles. Protocols were approved by the Minneapolis Medical Research Foundation Animal Care and Use Committee.

2.2. Immunization

The nicotine vaccine used was (±)3′-aminomethylnicotine (3′-AmNic) conjugated to the carrier protein recombinant *Pseudomonas* exoprotein A (rEPA) through a succinic acid linker [20]. This immunogen has been extensively evaluated in rats and is in Phase III clinical trials for smoking cessation [21–24] (personal communication, D. Hatsukami). Antibodies elicited by 3′-AmNic-rEPA in rats have a high affinity for nicotine (10–40 nM) and high specificity for nicotine; there is cross reactivity to the active but minor nicotine metabolite nornicotine, but <1% cross-reactivity with inactive nicotine metabolites including cotinine and nicotine-N-oxide, the endogenous nicotinic cholinergic receptor ligand acetylcholine, and a variety of related compounds [20]. Rats were immunized with an initial injection of 25 μg 3′-AmNic-rEPA in complete Freund's adjuvant i.p. followed by booster doses at 3 and 6 weeks in incomplete Freund's adjuvant. Experiments were performed 1 week after the final vaccine dose. Passive immunization, administered 24 h prior to smoke or nicotine exposure, was accomplished by i.v. infusion of the nicotine-specific monoclonal antibody Nic311, an IgG_1 kappa which has affinity and specificity for nicotine similar to that of antibodies generated by 3′-AmNic-rEPA in rats [25]. For active immunization studies, control rats received 25 μg of unconjugated carrier protein (rEPA) alone in adjuvant, and for passive immunization studies controls consisted of polyclonal nonspecific human IgG (Gammagard, Baxter Health Care, CA) which does not bind nicotine.

2.3. Immunologic assays

Serum Nicotine-specific IgG concentrations were measured by ELISA using 3′-AmNic-polyglutamate as the coating antigen to avoid detecting antibodies to the carrier protein of the vaccine, and serum standards with known nicotine-specific IgG concentrations to allow quantitation [22]. This assay is not affected by the presence of nicotine in serum or tissue samples. Serum or broncheoalveolar lavage (BAL) fluid nicotine-specific IgG and IgA levels were compared using antibody titers rather than concentrations because validated quantitative standards for nicotine-specific IgA concentrations were not available. Serum nicotine-specific IgG titers correspond closely with the measured serum concentrations [22].

2.4. Drug preparation and analysis

Nicotine bitartrate was dissolved in normal saline and pH adjusted to 7.4 with NaOH for administration. Nicotine concentrations are expressed as the base. Nicotine concentrations were measured by gas chromatography with nitrogen–phosphorus detection [26]. This method measures total nicotine (free nicotine as well as nicotine bound to antibody). Protein binding of nicotine in serum was measured by equilibrium dialysis [27]. Brain nicotine concentrations were corrected for brain blood content [22].

2.5. Cigarettes

3R4F research cigarettes (University of Kentucky) containing 13 mg nicotine/cigarette per assay in our lab, and a Federal Trade Commission method smoke yield of 0.8 mg nicotine and 9.2 mg tar were used. These yields are similar to commercial filter cigarettes.

2.6. Smoke exposure

The smoke exposure system has been described in greater detail elsewhere [19]. In brief, the system (TSE Systems, Bad Homburg, Germany) consisted of a computer-controlled rotary smoke generator that holds up to 10 cigarettes at a time and withdraws mainstream smoke using a syringe under the standard FTC conditions of 35 ml/puff withdrawn over 2 s. Smoke is delivered to a mixing chamber where it is diluted with air to the desired concentration and directed to either the nose-only exposure (NSE) or whole body exposure (WBE) units. The NSE exposure unit consists of a cylinder with 8 exposure ports to which rats were exposed by placing them in a restraint bottle with their nose at an exposure port. The WBE exposure unit is a 2.3 m^3 box. The smoke mixture and flow rate can be adjusted, based on real-time monitoring of in-line oxygen and carbon monoxide concentrations, to provide a range of smoke concentrations and resulting serum nicotine concentrations. The smoke concentration in the NSE unit reaches steady state rapidly, so that rats are exposed uniformly over the 10 min period. The smoke concentration in the larger WBE unit takes approximately 45 min to reach steady state, so that the actual exposure time for the 2 h WBE group was 2 h and 45 min which provided 2 h at the target smoke concentration. Smoke exposures were performed with unanesthetized rats. The mean smoke CO concentrations listed below are approximations because the maximum readout of the system is 300 ppm and concentrations occasionally exceeded this value.

2.7. Protocols

Rats were anesthetized with fentanyl/droperidol and a right jugular cannula placed for blood sampling. Experiments were performed 1–3 days later. Blood carboxyhemoglobin levels were not measured because these have already been reported using the same smoke exposure system in our lab as 7.6 ± 1.8% for a NSE that was considerably longer (45 min) than the 10 min exposure used here, and as 11 ± 0.8% for a 2 h WBE. These values are within the range reported for mid-day heavy cigarette smokers [28,29].

2.7.1. 10 min smoke NSE

Rats were acclimated to the restraint cylinders and NSE apparatus the day prior to study by simulating the exposure procedure but using room air rather than smoke. Rats were not anesthetized during smoke exposure. To study conditions similar to the smoking of 1 cigarette, which provides a serum nicotine concentration boost of 6–12 ng/ml [12,30] vaccinated rats were subjected to a 10 min NSE at a smoke flow rate of 12 L/min and smoke CO concentration of 235 ppm. Groups of 12 rats vaccinated with either 3′-AmNic-rEPA or unconjugated rEPA alone (controls) were exposed in this manner. Immediately after the 10 min exposure, rats were anesthetized with i.v. methohexital, blood was obtained through the jugular cannula, animals were decapitated and the brain was removed for analysis.

2.7.2. I.V. nicotine exposure

To compare the NSE exposure condition to a similar i.v. nicotine exposure, groups of 12 rats were anesthetized with droperidol/fentanyl, the right jugular vein was cannulated, and a 10 min infusion of either nicotine 15 μg/kg or saline was administered. This nicotine infusion rate was found in pilot studies to produce a mean serum nicotine concentration at the end of infusion of about 8 ng/ml, similar to that of the 10 min NSE. Trunk blood and brain were obtained after decapitation for measurement of nicotine and antibody levels.

2.7.3. 2 h smoke WBE

To provide a longer smoke exposure modeling more sustained smoking, groups of 12 rats vaccinated with either 3′-AmNic-rEPA or unconjugated rEPA alone were subjected to 2 h WBE at a flow rate of 70 L/min and a mean smoke CO concentration of 79 ppm. The target serum nicotine concentration was 20–30 ng/ml [31,32]. Sample collection was as described for the 10 min NSE exposure. The external catheter tip was capped during smoke exposure and cleaned thoroughly with alcohol before blood sampling.

2.7.4. Passive immunization

In addition to the jugular venous cannula, a femoral venous cannula was placed for Nic311 administration. Groups of 12 rats were anesthetized and received 36 mg/kg of Nic311 or nonspecific IgG control. This Nic311 dose has been shown to alter nicotine distribution to approximately the same extent as active immunization with 3′-AmNic-rEPA [33]. Rats were studied 1 day later, to allow sufficient time for distribution of Nic311 [25], by placing the unanesthetized animals in the WBE unit for 2 h with smoke parameters as noted above.

2.8. Sample collection

Serum and brain were obtained within 3 min of the end of each experiment and stored at −20 °C until assay. Broncheoalveaolar lavage (BAL) fluid was obtained, as a means of sampling pulmonary mucosal fluid, within 5 min by intubating the mainstem bronchus with a 13 gauge blunt needle, instilling 1 mL of saline into the mainstem bronchus while the rat was positioned nose-up, and withdrawing as much fluid as possible (0.8–0.9 mL) after 3–5 s while the rat was positioned nose-down at 30° [34]. Nicotine concentrations in lung tissue were not measured because the high blood content of lung (25% by weight), along with the very high serum nicotine concentrations in vaccinated rats, makes this level difficult to estimate [22].

2.9. Statistical methods

Results are presented as the mean ± SD. The means of measured values comparing vaccine and control groups were compared using one sided *t* tests. Two sided t tests were used for comparisons between the 10 min NSE and 10 min IV infusion groups. The relationship of serum antibody concentration to brain nicotine concentration was analyzed by linear regression, and the regression slopes were compared between the NSE and i.v. infusion groups by analysis of covariance. One vaccinated rat in the 10 min NSE protocol was excluded from analysis because of an error in sample handling. Two vaccinated rats in the 2 h WBE protocol were excluded from protein binding analysis due to sample contamination.

3. Results

3.1. 10 min exposures

3.1.1. 10 min smoke NSE

The serum nicotine-specific IgG antibody concentration in rats vaccinated with 3′-AmNic-rEPA was 280 ± 120 μg/ml, comparable to previous studies in rats with this vaccine (Table 1). The serum nicotine concentration in control rats immunized with rEPA alone was 7 ± 1 ng/ml, as intended to approximate the serum nicotine concentration boost produced by the smoking of 1 cigarette. Vaccination resulted in substantial retention of nicotine in serum (210 ± 80 ng/ml) compared to controls ($p < 0.001$) and a reduction in brain nicotine concentration of 90%, from 45 ± 14 ng/g in controls to 4 ± 1 in vaccinated rats ($p < 0.001$, Fig. 1). There was a significant inverse relationship between the serum antibody concentration and the brain nicotine concentration (Fig. 1). The mean serum nicotine-specific IgA titer was 24% that of the IgG titer (Fig. 2). Serum protein binding of nicotine, and BAL fluid nicotine concentrations, are not reported for either the 10 min smoke NSE or the 10 min i.v. nicotine exposure groups (Section 3.1.2) because many values were below the limit of quantitation (2 ng/ml) due to the low nicotine doses administered.

3.1.2. 10 min i.v. nicotine exposure

The serum nicotine-specific IgG concentration in rats vaccinated with 3′-AmNic-rEPA was similar to that of the 10 min smoke NSE group. The serum nicotine-specific IgA titer was substantial, with a mean value 25% of the concurrent nicotine-specific IgG titer (Table 1). The serum nicotine concentration in control rats was 8 ± 1 ng/ml, which achieved the goal of matching the end-of-exposure serum nicotine concentration to that of the 10 min smoke

Table 1
Serum nicotine-specific antibody concentrations and titers (mean ± SD).

	Vaccine protocols		Nic311	
	10 min NSE	10 min i.v.	2 h WBE	2 h WBE
Serum				
IgG concentration (μg/ml)	280 ± 120	270 ± 160	250 ± 140	230 ± 30
IgG titer × 10^3		160 ± 110	120 ± 60	
IgA titer × 10^3		40 ± 25	24 ± 12	
BAL				
IgG titer × 10^3	ND	ND	1.0 ± 1.0	ND
IgA titer	ND	ND	<0.2	ND

ND = not detected.

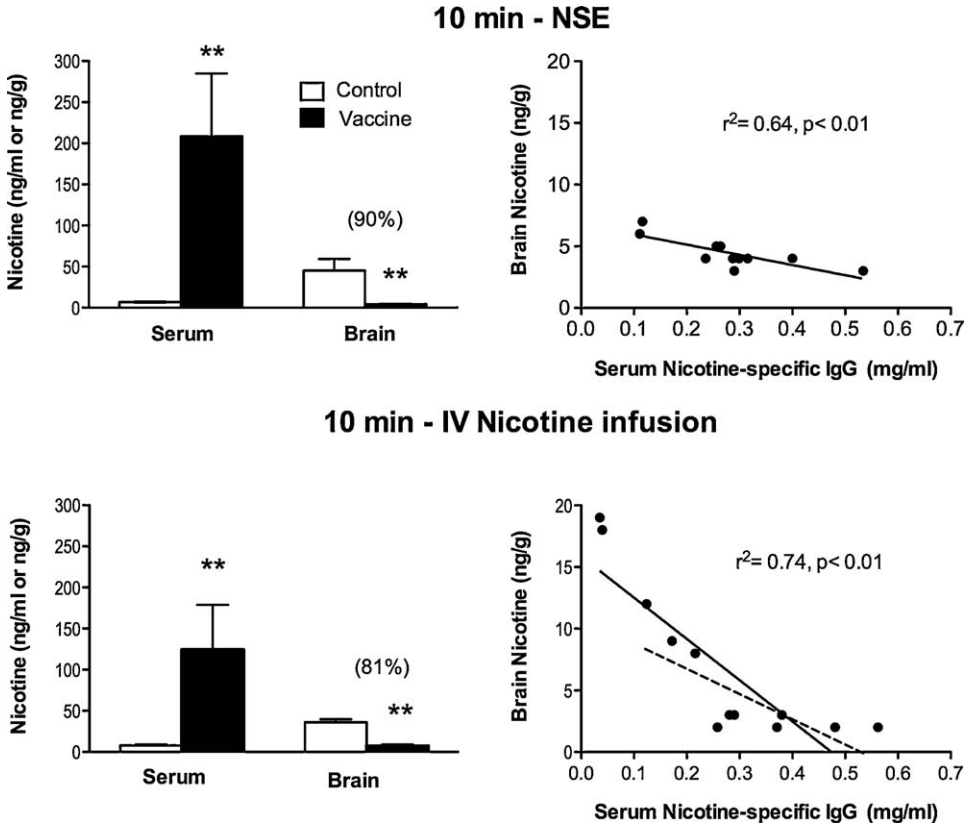

Fig. 1. 10 min exposures: serum and brain nicotine concentrations (left, mean ± SD) and the relationship between the serum nicotine-specific IgG concentration and brain nicotine concentration (right) in vaccinated rats receiving 10 min exposures to smoke (top) or i.v. nicotine alone (bottom). The percent reduction in brain nicotine concentration is shown in parentheses. Control rats (white bars) consist of rats immunized with unconjugated carrier protein. The solid regression lines include all values, and the broken regression line excludes two outliers with very low serum nicotine-specific IgG concentrations (r^2 and p values not shown for dotted line). Vaccination effects were generally comparable in the NSE and i.v. nicotine infusion groups. $^{**}p < 0.001$.

NSE. The effects of vaccination on serum and brain nicotine concentrations were generally similar to those observed in 10 min NSE group. Vaccination resulted in a substantial increase in serum nicotine concentration (120 ± 60 ng/ml) compared to control treatment ($p < 0.001$), and a reduction of brain nicotine concentration of 81%, from 36 ± 4 ng/ml in controls to 7 ± 6 in vaccinated rats ($p < 0.001$, Fig. 1).

3.1.3. Comparison of the 10 min NSE and 10 min IV infusion exposures

There was a significantly higher serum nicotine concentration in the vaccine group of the NSE exposure than the vaccine group of the 10 min IV infusion exposure ($p < 0.01$), but no significant differences in the serum nicotine concentrations in the control groups immunized with the protein carrier alone, or the brain nicotine concentrations in either the control or vaccine groups (Fig. 1). The slopes of the regression lines relating serum nicotine-specific IgG concentration to brain nicotine concentration differed between the NSE and infusion exposures (Fig. 1, solid regression lines, $p < 0.01$) but this difference was driven by two rats which had very low serum antibody concentrations. If these two rats were excluded from analysis (dotted regression line) there was no significant difference in the regression slopes between NSE and IV infusion exposures.

3.2. 2 h WBE exposures

3.2.1. 2 h smoke WBE; serum and brain nicotine and nicotine-specific antibody levels

The serum nicotine-specific IgG concentration in rats vaccinated with 3′-AmNic-rEPA was similar to that of rats in the 10 min exposure protocols (Table 1). The serum nicotine-specific IgA titer was appreciable with a mean value 21% of the concurrent nicotine-specific IgG titer, and a close correlation between the serum nicotine-specific IgG and IgA titers (Fig. 2). The serum nicotine concentration in control rats was 32 ± 8 ng/ml, as intended to approximate mid-day serum nicotine levels in a smoker. Vaccination resulted in significant nicotine retention in serum (790 ± 320 ng/ml) (Fig. 3). The reduction of brain nicotine concentration of 35%, from 190 ± 40 ng/ml in controls to 120 ± 60 ng/ml in vaccinated rats ($p < 0.01$) was smaller than the reduction seen in the 10 min exposure protocols. Vaccination substantially increased the serum protein binding of nicotine (Table 2).

3.2.2. 2 h smoke WBE; BAL fluid nicotine and nicotine-specific antibody levels

BAL nicotine-specific IgG titers were <1% of the corresponding nicotine-specific IgG titers (Table 1). The mean BAL nicotine concentration was increased by 230% in vaccinated rats compared to controls ($p < 0.001$), and there was a significant positive correlation between BAL nicotine and BAL nicotine-specific IgG concentrations (Fig. 4). The total amount of nicotine retained in the 1 ml of BAL fluid collected which was attributable to vaccination (the difference between the vaccine and control group means) was 0.01 μg or approximately 0.03 μg/kg. BAL fluid nicotine-specific IgA titers were below the limit of quantitation of the ELISA assay (<200).

3.2.3. 2 h smoke WBE with passive immunization

Serum Nic311 concentrations were in the same range as those of vaccine-generated antibodies in the 3 other protocols (Table 1). Changes in serum, brain and BAL nicotine concentration in passively immunized rats, compared to controls receiving nonspecific IgG, were very similar to those observed in the 2 h WBE vaccination protocol (Figs. 1 and 3). Changes in protein binding

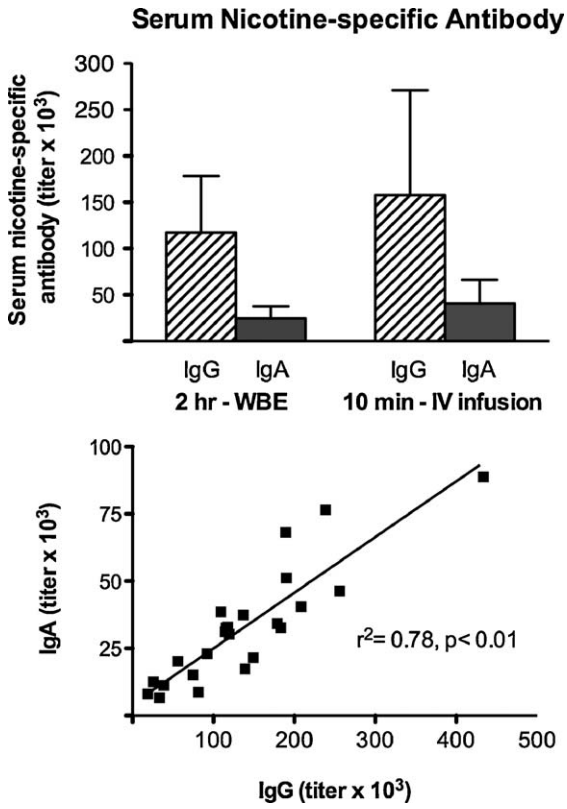

Fig. 2. Nicotine-specific IgG and IgA: serum nicotine-specific IgG and IgA titers in vaccinated rats after 2 h WBE to cigarette smoke or 10 min i.v. infusion of nicotine, mean ± SD (top) and the correlation between IgG and IgA titers (bottom). Values from both experiments were combined for the correlation analysis because rats were immunized using the same vaccination regimen and differed only in the nicotine dosing schedule.

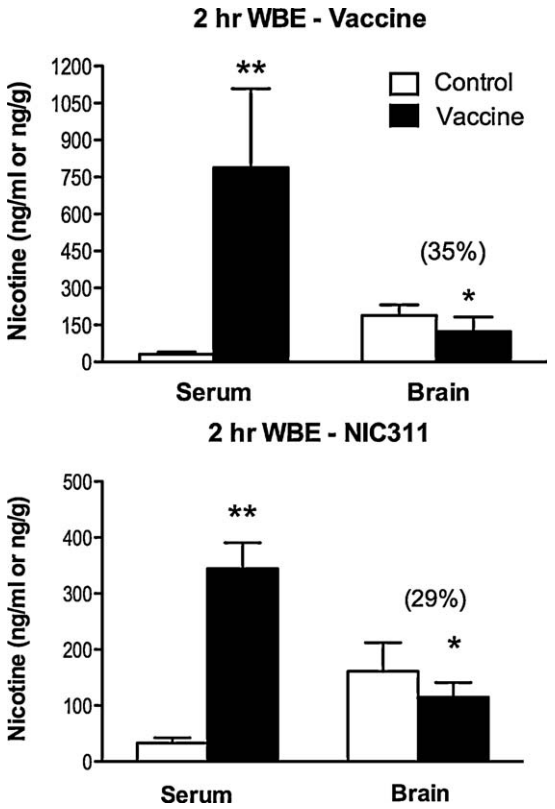

Fig. 3. 2 h exposures: serum and brain nicotine concentrations in rats receiving 2 h exposures to smoke after vaccination or passive immunization with Nic311. The percent reduction in brain nicotine concentration is shown in parentheses. The reductions in brain nicotine concentrations were comparable and less pronounced than seen after 10 min NSE (Fig. 1). $^{*}p < 0.01$ and $^{**}p < 0.001$.

parameters were also similar to those produced by vaccination, with a substantial increase in protein binding and reduction in the free (unbound) nicotine concentration in the Nic311 group (Table 2). The BAL fluid Nic311 titers were below the limit of quantitation of the ELISA assay (<200).

4. Discussion

This study used cigarette smoke exposure in an effort to capture some of the features of nicotine delivery to a cigarette smoker that are not provided by parenteral nicotine dosing. Rodent models of cigarette exposure have been used widely to study tobacco smoke toxicology, particularly carcinogenesis [13,14]. Much less attention has been directed at using smoke exposure systems to study nicotine pharmacology or pharmacokinetics, and most such studies have not measured serum or tissue concentrations of nicotine to determine how they relate to the exposures of cigarette smokers, or carboxyhemoglobin concentrations to assure lack of toxicity from carbon monoxide exposure. An exception is a NSE paradigm exposing rats to 10 pulses of cigarette smoke over 10 min which was used to model smoking topography and which found nicotine absorption from smoke to be rapid, and nicotine elimination to be comparable to that of nicotine administered i.v. [15,16]. Precise characterization of exposures may not be necessary for many types of smoke pharmacology studies, but is critically important when evaluating an intervention such as addiction vaccines which act by altering nicotine pharmacokinetics. The smoke exposure system used in this study has been characterized with regard to nicotine and carbon monoxide exposures over a range of clinically relevant smoke exposure conditions, allowing its use to more closely and quantitatively model the exposures of smokers [19]. Clinically relevant nicotine exposures can be obtained without excessive carboxyhemoglobin accumulation or overt evidence of behavioral toxicity. This type of well-defined exposure should be useful in studying nicotine vaccines, as well as other aspects of tobacco smoke pharmacology and the modifying effects of non-nicotine components of tobacco smoke.

The main finding of this study is that vaccination with 3′-AmNic-rEPA substantially altered the distribution to brain of nicotine delivered via cigarette smoke inhalation, with a reduction of 90% after a single 10 min exposure modeling the smoking of 1 cigarette, and a reduction of 35% after a 2 h exposure modeling a period of more sustained smoking. The smaller reduction after longer exposure is consistent with previously reported effects of this vaccine on nicotine administered i.v., which are greatest when the nicotine dose is low, when brain nicotine concentrations are measured shortly (1–25 min) after a nicotine dose, and when nicotine is delivered via bolus doses as compared to continuous infusion [35].

Table 2
Nicotine protein binding in serum (mean ± SD).

Protocol	Total nicotine (ng/ml)	Nicotine % bound	Unbound nicotine (ng/ml)
2 h WBE/vaccine			
Control	32 ± 8	12.2 ± 4.7	29 ± 8
Vaccine	790 ± 320**	96.9 ± 2.0**	22 ± 11
2 h WBE/Nic311			
Control	33 ± 9	8.2 ± 3.3	30 ± 9
Nic311	340 ± 50**	93.4 ± 1.8**	23 ± 7*

* $p < 0.05$ compared to control.
** $p < 0.001$ compared to control.

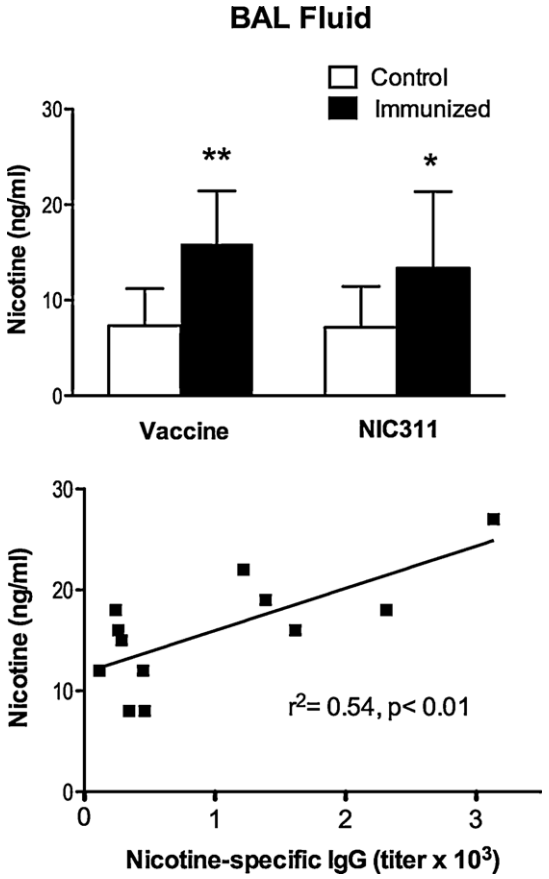

Fig. 4. BAL nicotine and IgG concentrations: (top) BAL nicotine concentrations after 2 h WBE to cigarette smoke in rats that were vaccinated or passively immunized with Nic311 (mean ± SD). (Bottom) Relationship of the BAL nicotine-specific IgG titer and nicotine concentration in vaccinated rats. BAL IgG titers for the Nic311 group were too low to quantitate. $^{*}p < 0.05$ and $^{**}p < 0.001$.

The similar serum nicotine concentrations in control groups, immunized with the unconjugated carrier protein, after 10 min NSE or 10 min i.v. infusion confirms that the nicotine dose delivered by 10 min NSE was in the target range of 0.015 mg/kg, similar to mg/kg estimates of nicotine uptake from a single cigarette by a smoker [36]. Vaccination substantially reduced nicotine distribution to brain whether nicotine was administered via smoke or by itself as an i.v. infusion, showing that the effects of vaccination on nicotine distribution are quite robust despite marked differences in route and manner of nicotine administration. These findings support the validity of the i.v. dosing paradigm in rats as a model of vaccine effects on cigarette smoking in humans. Several comparisons suggested that vaccination might be minimally more effective in the setting of NSE exposure than IV infusion exposure, but these were probably incidental because brain nicotine concentrations, the most important measure of efficacy, were comparable after these exposures.

The 2 h WBE exposure data are of interest because this exposure more closely approximates nicotine intake in someone smoking repeatedly throughout the day. The greater efficacy of vaccination in rats receiving the smaller (10 min) nicotine exposures compared to the 2 h WBE exposures, respectively achieving a brain reduction of 90% and 35%, can be put in perspective by estimating the relative molar ratios of drug to binding capacity of antibody. The total amount of nicotine-specific IgG in vaccinated rats can be estimated from the product of the measured serum concentration (mean of the vaccinated groups) and the reported steady state volume of distribution of IgG in rat (268 μg/ml × 125 ml/kg = 34 mg/kg = 0.22 μmol/kg), with 2 binding sites per IgG resulting in a binding capacity of 0.45 μmol/kg [37]. Using this estimate, the 10 min i.v. exposure (15 μg/kg = 0.093 μmol/kg) was equivalent to 21% of the estimated binding capacity of nicotine-specific IgG present. In this setting, there was a large excess of binding capacity compared to the nicotine dose. The nicotine dose delivered by the 2 h WBE is not known but in a previous study similar serum nicotine levels were produced by an i.v. nicotine infusion of 125 μg/kg/h (1.5 μmol/kg) infused over 2 h, which is equivalent to 330% of the estimated binding capacity of antibody [20]. The reduction in nicotine distribution to brain after 2 h WBE despite this excess of nicotine compared to antibody is of clinical interest because serum nicotine-specific IgG concentrations that have been achieved in vaccinated smokers in clinical trials (~50 μg/ml) are substantially lower than those routinely achieved in vaccinated rats (~250 μg/ml), yet vaccination appears to enhance smoking cessation rates [2,3].

A potential difference between inhaled and parenteral i.v. nicotine dosing is that absorption of nicotine via inhalation could be impacted by binding to pulmonary mucosal antibody. If present in sufficient amount, such antibodies could reduce or slow nicotine uptake by providing a barrier to absorption into blood. In this study both nicotine-specific IgG titers and nicotine concentrations were increased in the BAL fluid of vaccinated rats, and their values were positively correlated, showing retention of nicotine in pulmonary mucosal fluid by antibody. The fraction of the nicotine dose retained in pulmonary mucosal fluid by immunization cannot be calculated because the extent of recovery of pulmonary mucosal antibody in BAL fluid is not known. The absolute amount of nicotine retained in BAL fluid by immunization (the difference between immunized and control groups) was very low, equivalent to <0.1% of the 10 min smoke exposure dose and considerably less for the 2 h smoke exposures. Even assuming that recovery of pulmonary mucosal fluid in BAL fluid is inefficient, it is unlikely that nicotine binding in pulmonary mucosal fluid contributed appreciably to the kinetics of nicotine absorption from cigarette smoke.

The presence of nicotine-specific IgG in BAL fluid could reflect local production at the pulmonary mucosa or transfer of antibody from blood. Because nicotine was retained in BAL fluid to a similar extent by both vaccination and passive immunization with Nic311, nicotine-specific IgG must have been transferred from blood. Although nicotine-specific IgG was detected in BAL fluid after vaccination, Nic311 was not detected in BAL fluid after passive immunization. This is probably due to the lesser sensitivity of the ELISA assay for Nic311 compared to antibodies generated by vaccination, along with the somewhat lower levels of Nic311 in blood compared to nicotine-specific IgG in vaccinated rats.

The role of drug-specific IgA in mediating the effects of nicotine vaccines, or other addiction vaccines, is not known. In the current study nicotine-specific IgA was not detectable in BAL fluid after vaccination, indicating that there was no appreciable accumulation of nicotine-specific IgA in pulmonary mucosa fluid. This is not unexpected since parenteral vaccination is not typically a potent stimulus for mucosal antibody production [38]. Intranasal vaccination of mice against nicotine, which delivers immunogen to the lung as well as the upper respiratory tract, has been shown to reduce nicotine distribution to brain in mice. Serum and saliva nicotine-specific IgA titers were detected, but BAL titers were not examined [39]. Intranasal or direct pulmonary immunization might be of interest to further explore whether sufficient pulmonary mucosal nicotine-specific IgA levels can be produced in this manner to influence nicotine absorption [40,41].

In contrast to the lack of measurable nicotine-specific IgA in BAL fluid, nicotine-specific IgA titers in serum after vaccination were substantial with values 21-25% as high as the nicotine-specific IgG titers. Serum IgA titers resulting from parenteral vaccination

against nicotine have not been reported previously. Accurate estimates for the volume of distribution of IgA are not available to allow calculation of total body nicotine-specific-IgA content, but these appreciable titers suggest that nicotine-specific IgA could contribute to the binding of nicotine and effects of vaccination.

A limitation of this study is that passive inhalation of cigarette smoke by rats does not reproduce the pulsatile puffing and deep inhalation of cigarette smoking by humans. Pulsatile uptake of nicotine and delivery of nicotine to brain is widely regarded as important for the rewarding and reinforcing efficacy of nicotine, since more rapid delivery of drug to brain is in general more reinforcing than slow delivery [9,42]. However a recent human imaging study found that brain uptake of ^{11}C-nicotine from spiked cigarettes is damped by transit through the lungs [43]. Patterns of brain nicotine uptake were variable, with a pulsatile character clearly present in some subjects but less so or absent in others. Since all subjects were experienced smokers, puff-related pulsatile nicotine uptake into brain may not be essential to maintaining smoking behavior, or a necessary feature of a smoke inhalation model in rats.

In summary, vaccination with the nicotine immunogen 3′-AmNic-rEPA substantially reduced the distribution to brain of nicotine delivered via cigarette smoke inhalation. These data add validity to the i.v. nicotine dosing regimens used previously to model tobacco use in rats, and provide a means of studying the role of pulmonary antibody or other route-specific factors in modifying the pharmacokinetics of nicotine inhaled from cigarette smoke.

Acknowledgments

The 3′-AmNic-rEPA immunogen and rEPA carrier protein were gifts of Nabi Biopharmaceuticals. Internal standard for the nicotine assay was a gift from P Jacob (University of California, San Francisco). Supported by PHS grants DA10714, DA010714-13S1, T32-DA07097, and a Career Development Award from the Minneapolis Medical Research Foundation (MP).

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